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J. BEATTIE
J. KIRK (TREASURER)
J. D. BOYD
C. M. WEST
W. E. LE GROS CLARK (ACTING EDITOR)

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JOURNAL OF ANATOMY

A NOTE ON THE INTERPARIETAL GROOVE IN EGYPTIAN SKULLS

By L. R. SHORE

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I. INTRODUCTION

The purpose of this note is to draw attention to a certain groove which has been found to traverse the outer surface of the vault of the skull in a number of specimens derived from Ancient Egypt.

Fig. 1 presents photographs of two skulls showing the interparietal groove. The skull on the left, No. 3408, bears an antero-posterior groove which is defined by two ridges which lie closely together in front and diverge behind near the parietal foramina. Both in front and behind the ridges disappear as they merge into the general curved surface of the skull vault and the groove disappears. In the skull on the right in Fig. 1, No. 4325, the posterior ends of the parietal ridges diverge right and left in front of the parieto-occipital suture. The occipital bone and each parietal bone bears a ridge which follows the lambdoid suture from side to side, behind it, with the effect that paired parieto-occipital grooves join the interparietal groove and make a complex groove of Y form. In this skull, then, the interparietal groove is associated with paired parieto-occipital grooves.

The upper photographs in Fig. 1 show the depth of the grooves in each of these two skulls, which were suitably tilted before they were photographed.

II. MATERIAL

A large number of Egyptian skulls preserved in the Anatomy Museum at Cambridge are collectively known as the "Petrie" collection. In my survey of a small part of the whole collection, I took skulls as they came to hand from the store cases, with no more conscious selection than is implied by picking

out those which were fairly complete, and not in need of considerable reconstruction. It is hoped, therefore, that the skulls which provide the material for this note represent a fair sample. It seems that the skulls belong to all epochs from the Predynastic era to that of the XXVIth Dynasty. A group of 100 skulls is made up as follows:

Approximate epoch	No. examined	No. with the interparietal groove	Museum reference No. (see Tables I and II)
Predynastic	10	3	3430, 3552, 3570
Dynasties VI–XI	12	2	3407, 3408
„ X–XII	5	0	—
„ XVIII–XXVI	61	7	4262, 4289, 4299, 4305, 4325, 4343, 4399
Unclassified	12	0	
Totals	100	12	

It is notoriously difficult to "sex" skulls with confidence; therefore sex references which follow must be taken to represent approximations made to the best of my judgement with wide possibilities of error. The 100 skulls were made up by 55 males and 45 females, as I judge, and included 12 specimens equally divided between the sexes as regards the presence of the interparietal groove.

III. NOMENCLATURE

I have been unable to find a description of an external groove on the vault of the skull which corresponds to the interparietal groove other than that of Le Double, quoted below. In the absence of an accepted nomenclature I offer the name "Interparietal Groove" for the antero-posterior groove I have indicated. Le Double makes a reference to the "sulcus sagittalis externus" in describing a prelambdoid depression, writing as follows:

> Cette dépression prélambdoidienne insolite est representée tantôt par un sillon plus ou moins large et profond, situé sur la ligne médiane antéro-postérieure du crâne, et qui est généralement limité à la portion obélique de la synarthrose biparié-tale, mais qui peut comprendre toute la moitié postérieure de cette synarthrose, empiéter même sur le sommet du lambda (*Sulcus sagittalis externus* des anatomistes étrangers). Tantôt elle est constituée par une fossette de dimensions variables, plus ou moins excavée, ayant la forme d'un triangle dont le sommet regarde en avant et la base en arrière (*fovea prelambdoidea* des anatomistes étrangers). Il est très rare que la fossette prélambdoidienne soit hémilatérale, située à droite ou gauche de la suture biparietale, au niveau de l'angle postéro-supérieur de l'os du vertex.(1)

It seems that Le Double recognizes an antero-posterior groove on the skull, which may be limited to the region of the obelion. The mention of the triangular "prelambdoid" fossa is interesting, but there is no indication of the means by which the triangle acquires its third side. Unfortunately no explanatory figure accompanies Le Double's text, nor have I been able to trace his references to the writings of foreign anatomists.

To anticipate: I may state that descriptions of an interparietal groove both fore-and-aft and para-obelic in position and of a triangle follow in their places.

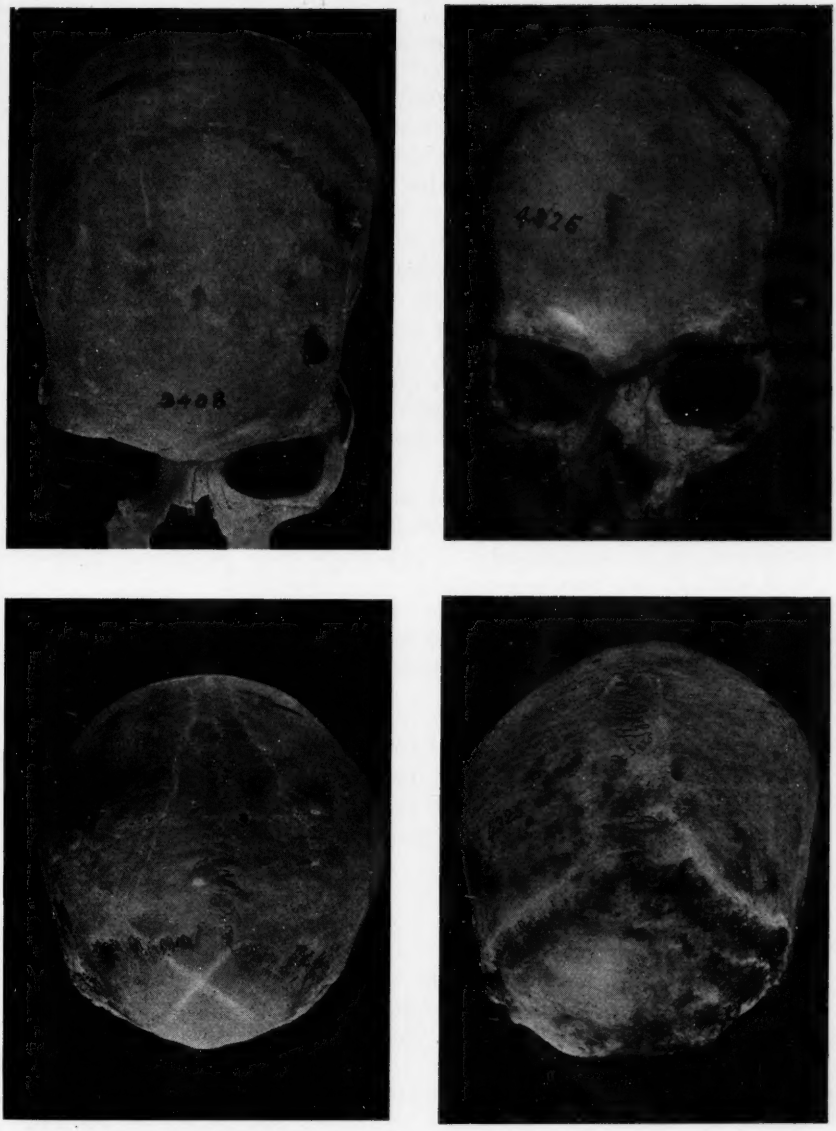

Fig. 1. Photographs of two Egyptian skulls viewed in two different positions. For each skull the lower view is the norma occipitalis, and the view above it is frontal and tilted so as to show the depth of the interparietal groove. In No. 3408, on the left, two ridges picked out lightly in white chalk can be seen on the parietal bones near the interparietal suture diverging from before backwards. The lower photograph on the right is of a second skull, No. 4325, with an interparietal groove joining paired parieto-occipital grooves: again the ridges are slightly emphasized by white chalk.

For the following reasons the name "interparietal" may perhaps be more acceptable than "sagittal".

In precise usage the word "sagittal" should be applied to a section or to a structure which occupies a position exactly antero-posterior, quite fore-and-aft and confined to the middle plane of the body; the superior sagittal sinus satisfies these conditions. As regards the interparietal groove: I have not seen a skull in which the groove was coextensive with the sagittal suture; in every skull the groove and the ridges which define it lay behind the coronal suture.

In the skull shown on the left in Fig. 1, the ridges bounding the groove are symmetrical, but diverge posteriorly. Even if "sagittal" were acceptable for this specimen, the features of the groove in the skull on the right would show that the term is inapt in general. In this second skull the groove extends posteriorly into two lateral grooves which are evidently parieto-occipital. Though it might be permissible to refer to "parieto-occipital" extensions of the interparietal groove, the same in connexion with a "sagittal" groove would be obviously inappropriate.

"Interparietal" may be criticized, but at least the groove is defined by ridges which are situated on the parietal bones, and lie between the parietal eminences. Every skull described in this paper has a fore-and-aft groove; 5 out of the 12 have parieto-occipital grooves in addition.

The area defined by the two parietal and the occipital ridges in the right-hand skull in Fig. 1 may correspond to the "prelambdoid triangle" of Le Double, but is more properly "lambdoid" in its position.

IV. VARIATIONS IN THE FORM OF THE INTERPARIETAL GROOVE

Fig. 2 shows outline drawings of three skulls in norma verticalis, made with the aid of the dioptograph while the skulls were fixed in the Frankfort horizontal position. The ridges which limit the interparietal groove are shown by thick interrupted lines.

The tracing on the left (No. 3408, see also Fig. 1) shows a groove which is long and corresponds to about three parts of the parietal arc. In the next skull, No. 4262, the tracing shows a much shorter groove bounded by much shorter ridges. The tracing on the right, No. 4343, shows a skull with a groove which is rounded and defined by curved ridges which are most divergent in the vicinity of the parietal foramina. This, no doubt, is the para-obelic form noted by Le Double.

Fig. 3 shows two tracings made in norma occipitalis, and with the ridges again distinguished by heavy interrupted lines. The tracing on the left, No. 3570, shows an interparietal groove which is widest above the lambda and is continuous with short parieto-occipital grooves on either side. That on the right, No. 4325 (also in Fig. 1), shows a strongly marked interparietal groove joining equally marked parietal-occipital grooves. Both these skulls have a somewhat depressed lambdoid area which, being defined by three

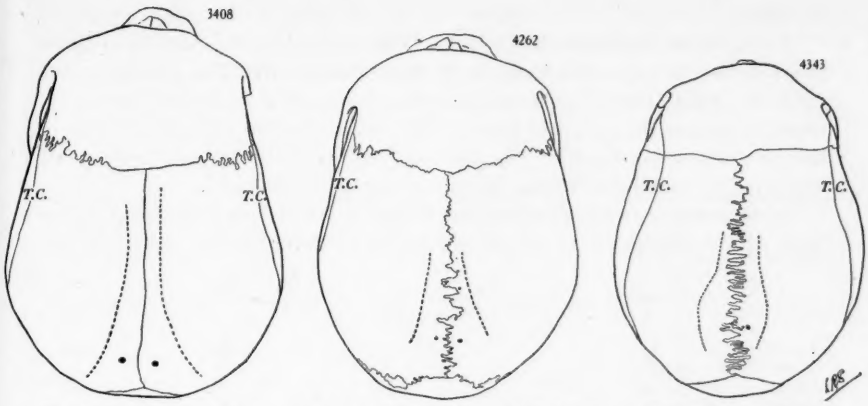

Fig. 2. Tracings made in true norma verticalis of three skulls by means of the dioptograph and showing three examples of the interparietal groove. In each tracing the ridges that define the groove are represented by heavy dotted lines. On the left is shown a long interparietal groove on the skull also shown on the left in Fig. 1. In the middle is a shorter groove, and on the right is a groove of somewhat elliptical form and para-obelic in position. The continuous line marked T.C. represents the temporal crest.

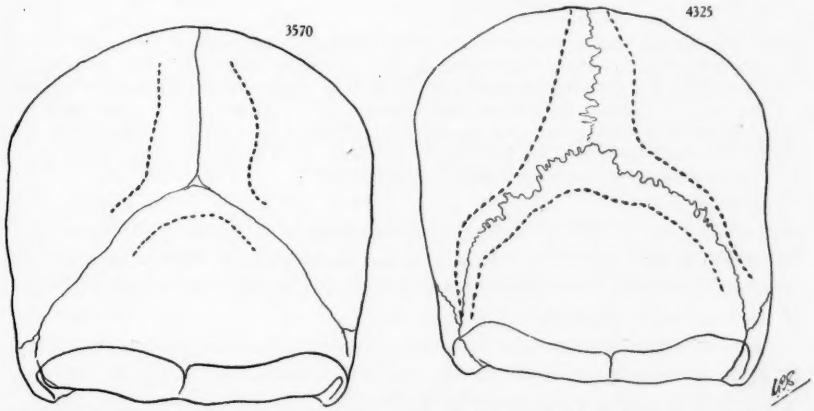

Fig. 3. Tracings of two skulls made in norma occipitalis by means of the dioptograph. The skull on the left shows a short interparietal groove joining two short parieto-occipital grooves. The skull on the right, also shown on the right in Fig. 1, has more extensive grooves both interparietal and parieto-occipital. In both these tracings a space is defined by the parietal and the occipital ridge which may be called the "lambdoid triangle".

ridges, might correspond with the triangular "prelambdoid" fossa of Le Double.

In Fig. 4 the tracing on the left, No. 3552, shows that the parieto-occipital grooves may be separated from the interparietal groove. The tracing on the right, No. 3430, shows that the triangle may be bounded posteriorly by a ridge which is not on the occipital bone at all, but is situated entirely in front of the parietal-occipital suture. In this case only does the triangle truly merit the name "prelambdoid"; the others are surely "lambdoid".

In the series of tracings reproduced in Figs. 2, 3 and 4 are to be found variations of the interparietal groove which include forms which are short and

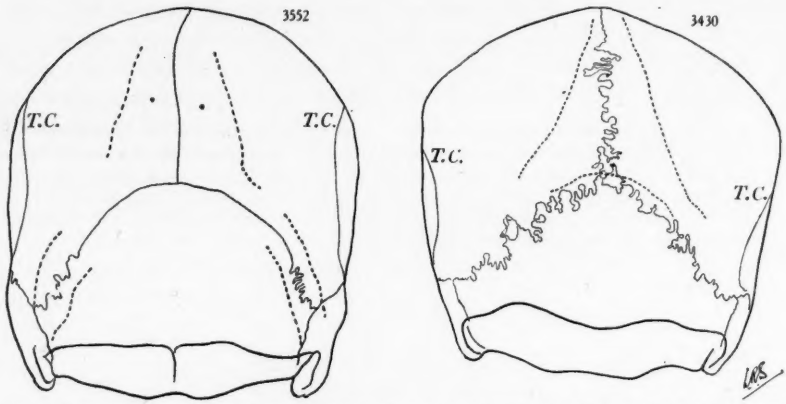

Fig. 4. Dioptograph tracings of two skulls in norma occipitalis. In the skull on the left, No. 3552, the interparietal groove is separated from the two parieto-occipital grooves. In the skull on the right, No. 3430, the interparietal groove and the "lambdoid" triangle are well marked. The ridge which limits the triangle posteriorly occupies an unusual position, being in front of the parieto-occipital suture and traverses the posterior ends of the parietal bones and not the occipital bone at all. Here the triangle is properly "prelambdoid". Again, the temporal crest is indicated by a fine continuous line marked T.C.

para-obelic, forms which are long and widening from before backwards, and forms which are associated with parieto-occipital grooves. In consequence of these gradations in size and form, it is assumed that only one essential feature of the skull vault is displayed in this series.

Table I gives measurements relating to the interparietal groove and to the 12 Egyptian skulls in which it was observed. Inferences as to the relation of the groove to the whole vault of the skull may be made from the measurements of the antero-posterior length of the parietal arc, and from the distance behind the point bregma of the anterior end of the groove.

The ages of the individuals from which the several skulls were derived are inferred from the data given by Harris (2) as to the average age at which synostosis takes place in different cranial sutures. In the tracings the cranial

sutures are usually shown as simple lines. It is not to be inferred that in these skulls with the interparietal groove the pattern of the sutures is unusually simple. It has not been observed that the sutural pattern differs from the normal in the way either of simplicity or of complexity.

Table I

Museum No.	Sex	Age	Length cm.	Breadth cm.	Distance from bregma cm.	Parietal arc cm.	References to figures and remarks
3407	F.	65?	5·7	5·0	5·2	12·7	Greatest breadth at obelion. Posterior parietal flattening
3408	M.	40–45	9·5	5·0	1·7	15·0	Figs. 1 and 2
3430	M.	Young adult	8·1	6·4	5·2	13·3	Fig. 4. Parieto-occipital extensions
3552	F.	40–45	5·8	5·8	7·2	13·0	Figs. 5 and 6. Parieto-occipital extensions
3570	M.	Young adult	7·7	4·0	6·5	12·0	Fig. 3. Parieto-occipital extensions. Posterior parietal flattening
4262	F.	„	5·1	4·2	3·8	12·2	Fig. 3. Posterior parietal flattening
4289	F.	„	6·5	3·5	2·2	10·7	—
4299	F.	„	9·0	7·7	3·5	12·5	Parieto-occipital extensions
4306	M.	55?	5·9	2·6	3·3	11·0	Greatest breadth at obelion
4325	M.	40?	8·0	4·3	3·8	11·8	Figs. 1 and 5. Parieto-occipital extensions. Posterior parietal flattening
4343	F.	Young adult	6·2	3·5	4·2	12·4	Fig. 3. Greatest breadth at obelion
4399	M.	„	8·0	4·0	4·0	13·8	—

V. THE RELATION OF THE INTERPARIETAL GROOVE TO THE TEMPORAL CREST

It does not seem probable that the ridges that define the interparietal groove have any relation with the temporal muscle. Within the arc of the temporal crest, i.e. the ridge by which the temporal fascia gains attachment to the skull vault, the bone is smoother and of finer texture than it is on the vault of the skull in general. It is within this arc that the temporal muscle has its origin, and outside it that the ridges which limit the interparietal groove are found.

Poirier & Charpy (3) mention paired ridges on the parietal bones lying more or less parallel to the sagittal suture. A drawing shows a pair of antero-posterior ridges above the level of the parietal eminences, which the text interprets as temporal crests occupying an unusually high situation. Though this interpretation may well be the true one, the drawing rather suggests to me the presence of an interparietal groove, though certainly of greater width than any I have seen.

In order to clear up this point I have marked the temporal crest in my

tracings in fine continuous line thus — T.C., and thus have indicated, as I believe, the arc that separates the area covered by scalp from that covered by temporal muscle.

It seems clear that the ridges which define the interparietal groove, whatever their nature may be, are not the temporal crests. Until an opportunity comes to examine a skull with an interparietal groove in the dissecting room, neither this relation nor any possible relation between the groove and some specialized feature of the epicranial aponeurosis can be investigated further.

VI. SECTION OF THE SKULL VAULT

Wedge-shaped segments were taken out of the vault of these skulls so that the interiors might be examined. In each skull one cut followed the transverse plane in which the interparietal groove seemed most conspicuous, and met a second cut which was posterior.

The surfaces photographed and shown in Fig. 5 are anterior surfaces of the wedges removed from the skull vault. From above downwards the sections lie thus: male and female showing interparietal groove, then normal male and female.

In every case the bone is notably thinned in the district of the groove, and this thinning may occur whether the sagittal suture is closed or not, and whether or not diploë be present in the place of the suture. The skull vault is not thicker at the ridges than in a normal skull at the same place; most of the thinning seems to be in the diploë.

In no case did survey of the interior reveal any abnormality of contour. There was no indication whatever of the position of the ridges or of the groove on the inner surface of the skull vault.

One skull out of the 12, N. 4399, was not cut because it seemed too fragile, but examination through the foramen magnum showed no abnormality.

One skull, No. 3408, has a uniform thickening of the whole vault which makes it massive and heavy. The diploë is continuous across the sagittal suture and seems to be duplicated, suggesting that some chronic inflammatory process, perhaps akin to Paget's disease, has been in operation. However, there was no change in the inner contour of the skull vault, and this pathological process has, I think, no relevance to the presence of the interparietal groove.

VII. THE INTERPARIETAL GROOVE AND LATERAL SKULL CONTOUR

It sometimes happens that a skull possessing an interparietal groove presents a corresponding change in its lateral contour.

In Fig. 6 are shown tracings of two skulls made in true norma lateralis. In each tracing the anterior end of the interparietal groove is indicated by an arrow perpendicular to the vault. It can be seen that the posterior parietal part of the vault is flattened in the skull on the left, but not to a corresponding extent in that on the right.

Fig. 5. Photographs of sections through the vault of the skull made in order to show the interparietal groove. In each case a wedge of bone was taken out of the skull vault and the anterior surface of the wedge is shown in the photograph. The uppermost section traverses the deepest part of the interparietal groove of a male skull. The next section is similar, but of a female skull. The two lower sections are controls from normal male and female skulls cut in positions exactly corresponding to those shown above in Nos. 4325 and 4262 respectively. It will be noted that there is no indication of the interparietal groove or of the parietal ridges on the inner surface of skulls No. 4325 or No. 4262. The skull vault, and particularly the diploë, is thinned on either side of the interparietal suture.

It is perhaps worthy of remark that this flattening is not most conspicuous where the interparietal groove is deepest or longest. The skull on the left in Fig. 6, No. 3570, has a shorter interparietal groove than has the skull on the right, No. 3552, for this one has parieto-occipital extensions also, but presents less flattening in lateral contour.

More commonly it seems that the interparietal groove gives no indication of its presence from the lateral contour of the skull. Out of 12 skulls with the interparietal groove no more than 4 showed posterior parietal flattening (*vide* Table I).

VIII. THE INTERPARIETAL GROOVE AND SKULL FORM

A glance at the tracings in Fig. 6 will show the reader at once that the interparietal groove, associated with the parieto-occipital groove or not, is

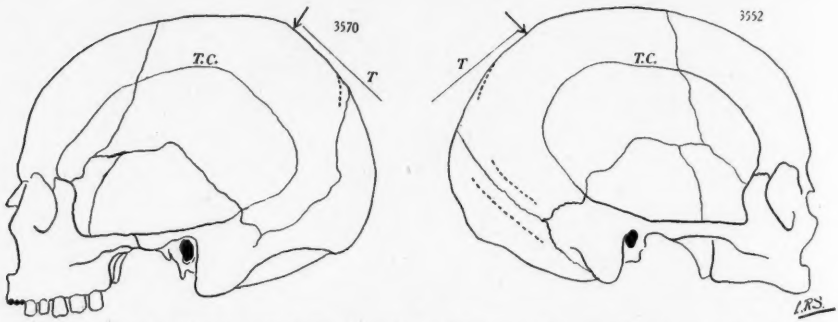

Fig. 6. Two lateral tracings of skulls possessing an interparietal groove. The arrow points to the anterior end of the groove which is shown in part in heavy dotted line. The line *T* is drawn tangential to the curve of the skull vault at the anterior end of the groove. It will be seen that the skull on the left, No. 3570, has a posterior parietal flattening which corresponds to the interparietal groove. In the skull on the right, No. 3552, flattening in the posterior parietal region is much less marked, and does not correspond to the interparietal groove so closely. Again, the temporal crests are indicated by fine continuous lines marked T.C.

not limited in its occurrence to any special type of skull. The skull on the right, No. 3552, is long and low; that on the left, No. 3570, is shorter but more lofty; in fact, the breadth-length ratio is 74·6 for No. 3570 and 70·4 for No. 3552. The ratio of basi-bregmatic height to length for the same two skulls is about 73·7 for No. 3570 and 70·8 for No. 3552. (The base of No. 3570 was damaged, so basi-bregmatic height becomes an approximation.)

I have made measurements of maximum length, of maximum breadth and of basi-bregmatic height in 100 skulls, and from these measurements I have calculated the breadth-length and the height-length ratios expressed as percentages. The breadth-length ratio varies from 70·4 to 79·2; the height-length ratio from 69·5 to 76·5.

In Table II are set out details of measurements of the skulls which have

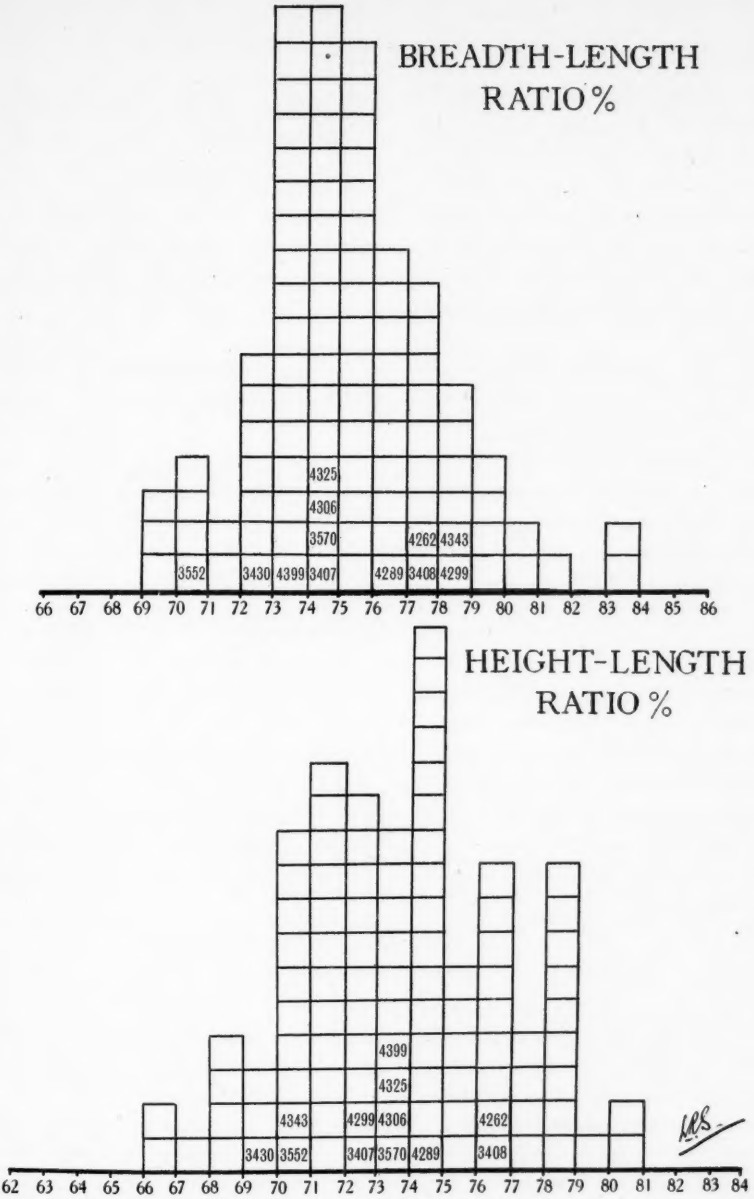

Fig. 7. These two graphs show the dispersion of the 12 Egyptian skulls possessing the interparietal groove in a group of 100, relative to the breadth-length and the height-length indices. The abscissae denote the values of the two indices expressed as percentages. The ordinates by squares of equal size represent the number of skulls whose indices for breadth-length or height-length fall between any two of the several numbers marked on the abscissae. The skulls which possess an interparietal groove are indicated by their Museum numbers written within their appropriate squares. It is inferred from the distribution of these numbers that there is no real relation between the presence of the interparietal groove and skull form as represented by either the breadth-length or the height-length index.

the interparietal groove together with the calculated ratios of breadth and height. The dispersion of these ratios in the group of 100 may be seen in graphic form in Fig. 7. In these graphs abscissae of equal length represent the breadth-length or the height-length ratios. The ordinates indicate the number of skulls found in the series whose breadth-length or height-length ratios lie between each pair of numbers in the abscissae. Each skull is indicated by a square of equal size, and every one that has an interparietal groove is indicated in its appropriate position by its Museum number.

Table II. *Measurements and indices of Egyptian skulls possessing the interparietal groove*

Museum No.	Sex	Estimated capacity in c.c.	Length	Breadth	*B/L* (%)	Height	*H/L* (%
3407	F.	1280	17·6	13·1	74·4	12·8	72·7
3408	M.	—	18·5	14·3	77·3	14·1	76·2
3430	M.	1427	18·8	13·6	72·2	13·1	69·5
3552	F.	1306	18·5	13·0	70·4	13·1	70·8
3570	M.	1317	18·2	13·6	74·6	*c.* 13·4	*c.* 73·7
4262	F.	1245	17·6	13·6	77·2	13·5	76·8
4289	F.	1412	16·9	13·0	76·8	12·5	74·0
4299	F.	1310	17·7	13·8	78·0	12·8	72·4
4306	M.	1396	17·9	13·3	74·3	13·3	73·2
4325	M.	1355	18·1	13·4	74·0	13·2	73·0
4343	F.	1397	16·9	13·2	78·2	11·9	70·3
4399	M.	1356	18·1	13·3	73·5	13·3	73·3

It seems from these two graphs that there is no real relation between the presence of the interparietal groove on the one hand, and the proportional height or length on the other, as expressed by two common indices.

The capacities of the several skulls set out in Table II have been calculated from formulae (attributed to Pearson) given in Martin's *Lehrbuch der Anthropologie*, which runs as follows:

Male $\quad C = 524{\cdot}6 \text{ plus } 0{\cdot}000266 \times L \times B \times H.$

Female $\quad C = 812{\cdot}0 \text{ plus } 0{\cdot}000156 \times L \times B \times H.$

(where H = basi-bregmatic height).

The capacities of the male skulls with interparietal grooves range from 1367 to 1407, and average 1389 c.c.; those for the female skulls range from 1226 to 1317, and average 1269 c.c.

Six unselected male skulls from the same source range in capacity from 1385 to 1566, and average 1470 c.c. Six unselected female skulls range from 1246 to 1345, and average 1290 c.c. It can only be remarked that the female skulls with the interparietal groove seem to conform very nearly with the unselected controls in their average capacity, while the male skulls with the interparietal groove have an average capacity rather below that of the controls.

No estimate of capacity is offered for No. 3408. As already stated, this

skull is the subject of some such change as Paget's disease with considerable but uniform thickening of the skull vault; and it seems likely that this change would invalidate any estimate of capacity based on external measurements.

IX. DISCUSSION

It is very difficult to offer an explanation of the occurrence of the interparietal groove in Egyptian skulls.

First, it is clear that it is not a sex character. Secondly, it has been demonstrated that the groove and its defining ridges have no relation with the temporal crest or with the temporal muscle. Thirdly, it does not seem that the presence of the groove has any relation with height, length or capacity of the skull.

Artefact is improbable, if only for the reason that artifice seldom fails in the production of an extreme and unequivocal effect, whereas the interparietal groove varies in a short series between the state in which the groove is very strongly marked, and that in which the groove is small and somewhat inconspicuous. A fore-and-aft band might perhaps produce such a groove as we are considering, but why should its effect be confined to the posterior part of the skull vault? Wider search might show that the groove is not always post-bregmatic in position, but may extend from the frontal region; but there is no sign of a secondary deformity suggesting a circular band to fix the first. For the sake of comparison I have examined a series of skulls from East Anglia, and have found on a number of these skulls specimens of the interparietal groove which are entirely comparable with those from Egypt. I have, however, not observed the parieto-occipital groove in East Anglian skulls.

This finding in the skulls of East Anglia makes it difficult to interpret the groove as some racial peculiarity. It was less surprising to find interparietal grooves in the casts of two skulls discovered by Leakey in Kenya, or to note the same feature in a photograph of a skull from Kerma reproduced in a paper by Collett(4), because these examples are certainly derived from the African continent, as are the Egyptian specimens. The discovery of some obscure racial trait in common seemed possible before the finding of the same feature in East Anglian skulls. It seems, then, that the interparietal groove is neither an artefact nor a race character.

The East Anglian skulls which I examined came from the fen district of Burwell, near Newmarket. These skulls have not resisted the wear and tear of time nearly so well as the Egyptian. Indeed, the Burwell skulls are so eroded, and in many cases so much reconstruction has been necessary, that I might have overlooked the interparietal groove but for experience in handling Egyptian skulls.

It remains to consider the possibility that the interparietal groove may have a developmental significance.

By common concensus of opinion the membranous roof of the skull in the

position of the posterior fontanelle becomes replaced by bone which grows from the two parietal and the supra-occipital centres of ossification, though the subsequent growth and "filling out" of the bones is undescribed. Phases of growth and of stasis, or perhaps more properly of consolidation, may occur at different times in the bones that bound the posterior fontanelle. If it could be shown that such is the case, it might be suggested that the ridges that mark the parietal and the occipital bones represent the high-water marks of certain tides of growth. For some reason the usual process by which tide marks are smoothed out and rendered inconspicuous has failed. In this respect, the thinning of the skull vault in the floor of the interparietal groove at least is a positive finding.

X. SUMMARY AND CONCLUSIONS

In a collection of skulls of ancient Egypt an antero-posterior groove was found marking the exterior of the skull vault defined by ridges lying on either side of the interparietal suture. This groove may widen as it is traced posteriorly, or it may be limited to the district of the obelion. The groove may meet paired parieto-occipital grooves and take on a **Y** form; in this case a lambdoid triangular depression may be associated with it.

Neither the groove nor the ridges give any trace of their presence on the interior of the skull, but section shows thinning in the groove, particularly of the diploë. The presence of the interparietal groove seems to have no relation to the form of the skull as expressed by the common breadth or height indices. It is possible that the presence of the groove is due to some failure of development of the bones that surround the lambdoid region. The occurrence of the interparietal groove is not confined to the skulls of Egyptians, but has also been noted in those of East Anglia.

XI. ACKNOWLEDGEMENTS

I wish to express my best thanks to Dr W. L. H. Duckworth for the many occasions upon which he has given me his time in order to discuss topics of Anatomy and of Anthropology, including the interparietal groove; and for many acts of kindness. It was Dr Duckworth who first showed me the interparietal groove in the Burwell skulls.

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A CONTRIBUTION TO THE STUDY OF THE MOVEMENTS OF THE TONGUE IN ANIMALS, WITH SPECIAL REFERENCE TO THE CAT

By SHAFIK ABD-EL-MALEK, M.B., B.Ch.

The Histological Laboratory, Physiology Dept., Cairo

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INTRODUCTION AND LITERATURE

The intricate interlacement of fibres of the intrinsic muscles of the tongue and hence the difficulty, or rather the impossibility, of throwing single muscles into contraction has been the cause of the prevailing uncertainty about the individual action of each of this group of muscles. It is not therefore improbable that some of the movements of the tongue are attributed to a certain muscle, when in reality they are affected by another. There is evidence in the literature that deviation of the tip of the tongue, during protrusion of this organ, is produced by the genioglossus muscle. This assumption seems to have originated from clinical observations in cases of injury either of a hypoglossal neurone, as may be seen in bulbar paralysis, or in pathological lesions of a corresponding upper motor neurone. When from similar causes paralysis of one side of the tongue follows, whether it is of the upper or of the lower motor neurone type, the tongue, on protrusion, deviates towards the paralysed side. Both anatomists and clinicians are of opinion that this deviation is produced by the unopposed action of the genioglossus muscle of the healthy side (Gray, 1935; Cunningham, 1937; Piersol, 1931).

Bennett (1935) was the first who justifiably refuted this conception. He found that stimulation of the trunk of the hypoglossal nerve in a series of

animals (monkey, dog, cat, rabbit and rat) results in a slight protrusion of the tongue with deviation of its tip towards the stimulated side. Stimulation of the distal part of the hypoglossal nerve leads to deviation of the tongue to the opposite side. This terminal branch—as the author calls it—is wholly distributed in the intrinsic muscles of the same side. The author therefore suggests that the intrinsic muscles of the tongue, and not the genioglossus, are those concerned with deviation of the tongue to the opposite side.

This investigation is carried out with the aim of properly understanding the individual action of the lingual muscles, and the part which each takes in the production of the different movements of the tongue.

METHODS AND RESULTS

Two methods of study were used in this investigation. The first was an experimental one in which movements of the tongue produced through stimulation of either the trunk or a branch of the hypoglossal nerve were observed. In the second, the musculature of the tongue was studied both anatomically and histologically.

(*a*) Experimental observations

Experiments were first performed on cats—fifteen of them were used. The results obtained were confirmed in six dogs and ten rats. Cats were anæsthetized with an intraperitoneal injection of a dose of dial (0·40–0·50 c.c. per kg. body weight). Dogs were given an intravenous injection of chloralose (0·10 g. per kg. body weight), while rats were given urethane intraperitoneally (0·05 g. per kg. body weight). The hypoglossal nerve was dissected and followed from its exit from the cranium towards its termination in the muscles of the tongue. The branches of supply from the nerve to these muscles were also exposed. As can be seen from Text-fig. 1 (which is a diagrammatic picture of the distribution of the hypoglossal nerve in the tongue of the cat), the trunk of the nerve after giving a few branches to the styloglossus, hyoglossus and genioglossus muscles, divides into a small lateral and a much larger medial division. The lateral division supplies the styloglossus, the hyoglossus, and the inferior longitudinal muscle. In its proximal part the medial division gives few collaterals to the inferior longitudinal and genioglossus muscles. Distally, it becomes entirely destined for the supply of the intrinsic lingual muscles. *A* and *B* are points at which the nerve was stimulated. *A* lies proximally on the trunk of the nerve, while *B* lies just distal to the exit of all its branches to the extrinsic muscles. Stimulation, therefore, of the intact nerve at *A*, results in contraction of the tongue muscles as a whole. When, on the other hand, the medial division is sectioned just above *B* along with section of the branches from the lateral division to the inferior longitudinal muscle, stimulation at *A* led to contraction of all the extrinsic muscles, while stimulation at *B* led to contraction of only the intrinsic ones (see Text-fig. 1).

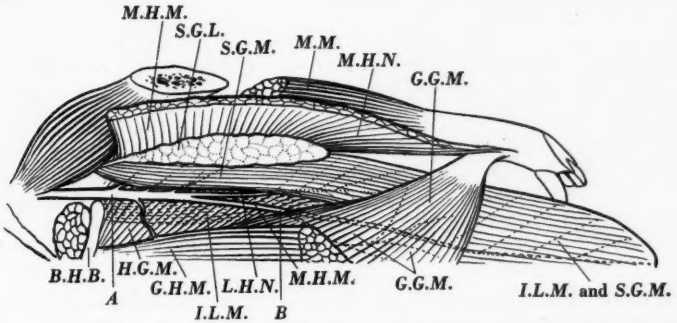
(i) *Experiments which result in contraction of the tongue muscles as a whole*

Experiment I.

The right hypoglossal nerve in the cat is exposed along its course, as is shown in Text-fig. 1. The nerve is stimulated at *A* with a faradic current of a medium strength. The following results were obtained (see Text-fig. 2):

(1) The tongue is protruded forwards and pulled towards the floor of the mouth.

(2) The dorsum of the right half of the tongue becomes concave from side to side.



Text-fig. 1. A diagrammatic illustration of the muscles of the tongue in the cat, as dissected from its ventral surface, as well as the distribution of the hypoglossal nerve to these muscles. *A* is a point on the trunk of the hypoglossal nerve proximal to the exit of all its branches to the lingual muscles. *B* is a point on the medial division of the hypoglossal nerve, proximal to the exit of all its branches to the intrinsic muscles.

(3) The tip of the tongue is deviated towards the stimulated side and turned caudally.

On splitting of the tongue in the middle line in some of the animals, the same findings were obtained.

Neither section of the hypoglossal nerve on the left side, nor section of the lingual and glossopharyngeal of the same side interferes with the results.

Experiment II.

The two hypoglossal nerves were exposed in the neck. Their trunks were approximated together and were both stimulated at *A* by one pair of electrodes. The following results were obtained:

(1) The tongue is protruded forwards, withdrawn to the floor of the mouth, and its dorsum became concave from side to side.

(2) The tip of the tongue is turned downwards without deviation.

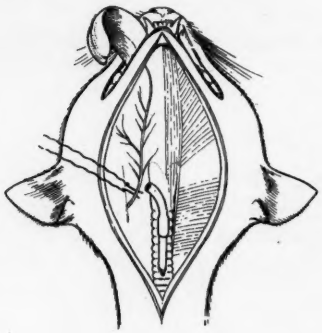
Experiment III.

The tongue was split in the middle and the two hypoglossal nerves were both exposed and stimulated at *A* as in Exp. II (see Text-fig. 3). The following results were obtained:

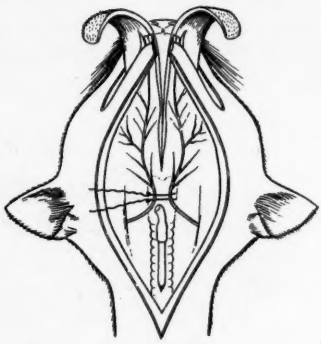
(1) Each half of the tongue was protruded outwards and pulled to the floor of the mouth.

(2) The dorsum became concave from side to side.

(3) The two tips turned downwards and diverged from each other in shifting to the corresponding side.



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. A diagram which shows the result of stimulation of the hypoglossal nerve trunk at *A*. The tongue deviates to the same side with its tip turned caudally. See Exp. I.

Text-fig. 3. The tongue is split in the middle line, and the two hypoglossi trunks are stimulated. The two halves of the tongue protrude and diverge from each other, and each half shifts to its corresponding side. The two tips are turned caudally. See Exp. III.

(ii) *Experiments which result in contraction of the extrinsic muscles only*

Experiment IV.

The right hypoglossal nerve, after being exposed as before, is divided at *B*. The branches from its lateral division to the inferior longitudinal muscle are also cut. Stimulation at *A*, which reaches only the extrinsic muscles of the tongue—not the intrinsic ones—gave the following results:

(1) The tongue is protruded to a lesser degree than when all the lingual muscles were in action.

(2) The tongue is pulled to the floor of the mouth and its dorsum showed a side to side concavity.

(3) The tip of the tongue deviates to the same side; the deviation is more pronounced than in Exp. II.

(4) The tip is turned downwards.

On comparison of the results of this experiment with those of Exp. I, it will be natural to conclude that, generally speaking, the intrinsic muscles, or at least some of them, do take part in protrusion of the tongue, act as opponents to the extrinsic muscles in homolateral deviation, in turning the

tip either upwards or downwards, and that apparently they take no part in pulling the tongue to the floor of the mouth.

Experiment V.

The two hypoglossal nerves are cut at *B*. The branches of each nerve to the inferior longitudinal muscle are also cut, both are simultaneously stimulated at *A*. The following results were obtained:

(1) Protrusion of the tongue is more than that obtained in Exp. IV.

(2) The tongue is strongly pulled to the floor of the mouth. Its anterior two-thirds become concave from side to side. Its tip turns caudally.

(3) No deviation of the tip occurs.

The absence of deviation in this case is apparently due to the antagonistic action of the muscles of one side on those of the opposite side. This leads in turn to the reinforcement of protrusion of the tongue and its withdrawal to the floor of the mouth. The tip of the tongue is partially turned downwards as this is usually helped by the intrinsic muscles. The validity of this conclusion finds justification in the following experiment.

Experiment VI.

The tongue is split in the middle line and the experiment proceeded with exactly as in Exp. V. The following results were obtained:

(1) Slight protrusion of the two halves of the tongue.

(2) Deviation of each half of the tongue to the corresponding side, with tip turned downwards.

(3) Pulling of each half to the floor of the mouth.

The individual action of the extrinsic muscles was studied in a series of animals through independent stimulation of the nerve supply to each muscle after exclusion of the intrinsic muscles by division of the hypoglossal nerve at *B*. It was found that:

(1) On stimulation of the nerve to the hyoglossus, the root of the tongue was pulled slightly downwards and retracted.

(2) On stimulation of the nerve to the styloglossus, the tongue was raised from the floor of the mouth, pulled postero-laterally (in a postero-lateral direction), and the tip was turned slightly downwards.

(3) The stimulation of the nerve to the genioglossus muscle resulted in:

(*a*) More protrusion of the tongue than in Exp. IV owing to exclusion of the action of hyoglossus and styloglossus.

(*b*) Pulling of the tongue to the floor of the mouth.

(*c*) A side to side concavity of the anterior two-thirds of the dorsum of the tongue.

(*d*) Deviation of the tip to the same side.

(iii) *Experiments which result in contraction of the intrinsic muscles*

Experiment VII.

The right hypoglossal nerve is exposed as usual, divided just above *B*, and stimulated distal to the point of sectioning. The following results were obtained:

(1) The tongue is protruded to a larger extent than in Exp. I.

(2) It becomes narrower and is flattened.

(3) Its tip deviates to the opposite side and is turned downwards—or ventroflexed.

Experiment VIII.

Exp. VII is repeated and the nerves to the inferior longitudinal muscle are cut in addition, giving the following results (see Text-fig. 4):

(1) The tongue is protruded exactly as in Exp. VII.

(2) It becomes narrower and is flattened.

(3) Its tip deviates to the opposite side but it is turned upwards or dorsiflexed.

Experiment IX.

Repetition of these last two experiments after splitting of the tongue in the middle line leads to the same results.

Experiment X.

The two hypoglossal nerves were exposed as before, divided as in Exp. VIII—with nerves to inferior longitudinal muscles cut—and stimulated simultaneously at *B*. The following results were obtained:

(1) The tongue is protruded.

(2) Its tip is turned upwards.

(3) There is no deviation.

Experiment XI.

Exp. X was repeated with the tongue split in the middle line, and the two nerves were stimulated simultaneously as in Exp. VIII, giving the following results (see Text-fig. 5):

(1) The two tips are protruded.

(2) They are turned upwards—dorsiflexed.

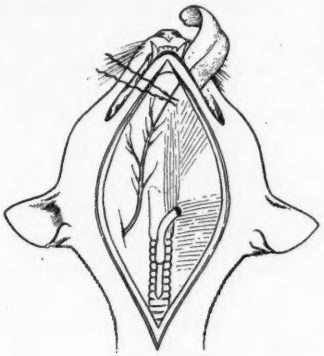
(3) They cross each other in this contralateral deviation.

Experiment XII.

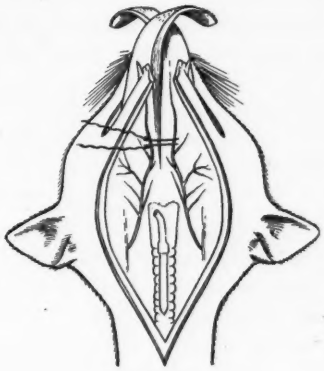
The right hypoglossal nerve is exposed as usual and divided just above *B*, and nerves to inferior longitudinal muscle are cut. The tongue is split in the middle line. The nerve is stimulated at *B*, and the movements of the tongue

observed. The fibres of the superior longitudinal muscle were cut transversely at a point just posterior to the middle of the length of the muscle, i.e. just posterior to its thickest part. The hypoglossal nerve was stimulated at *B*, and the following results were obtained (see Text-fig. 6):

(1) The right half of the tongue is protruded to a greater extent than when the same stimulation was applied before division of the muscle.



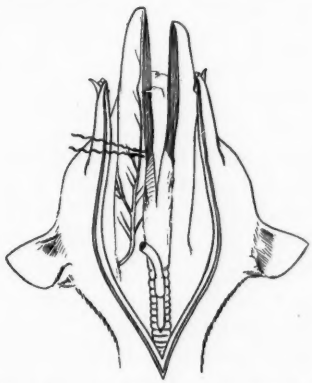
Text-fig. 4.



Text-fig. 5.

Text-fig. 4. This diagram illustrates the result of stimulation of the terminal branch of the hypoglossal nerve at *B*, i.e. its medial division. The tip protrudes as well as deviates to the opposite side, and turned rostrally in addition. See Exp. VIII.

Text-fig. 5. The tongue is split in the middle line, and the two terminal branches of the hypoglossi are stimulated. The two halves of the tongue cross each other in a contralateral deviation, and both are turned upwards and protruded as well. See Exp. XI.



Text-fig. 6. In this diagram the tongue is split in the middle line, and the superior longitudinal muscle is cut across at about its middle at the point *C*—in order to exclude its action. The terminal branch of the hypoglossal nerve of the corresponding side is stimulated, the tip of the tongue protrudes, but in a forward direction without deviation, and is turned neither rostrally nor caudally. See Exp. XII.

(2) It becomes narrowed and flattens.

(3) The tip is turned neither upwards nor downwards.

(4) There is no deviation at all.

On further experimentation it was found that transverse section of the superior longitudinal muscle at places other than that above mentioned causes a partial disappearance of deviation of the tip of the tongue on stimulation of the nerve at *B* dependent on the amount of muscle fibres injured.

It is therefore natural to conclude that the superior longitudinal muscle is the principal one concerned with deviation of the tip of the tongue to the opposite side, and turning of the tip upwards. Inferior longitudinal muscle is concerned with turning the tip of the tongue downwards. The transverse muscle both protrudes and narrows the tongue, while the vertical one flattens it.

(*b*) Morphological studies

The description of both the membranous and muscular structures of the tongue is the result of both macro- and microscopic studies of this organ.

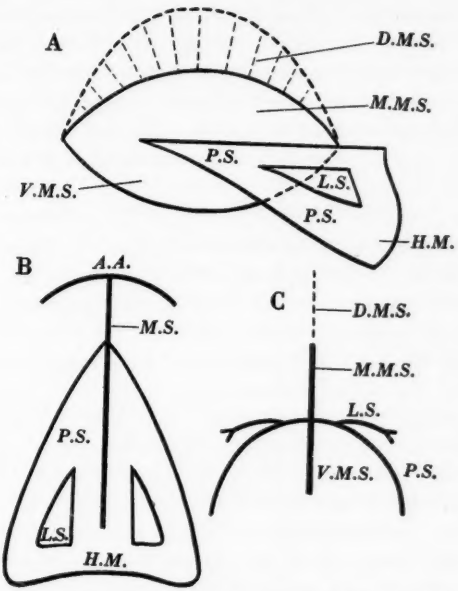
The observations here recorded are those found in the cat, supported by similar findings in the tongue of the rat.

For the sake of a better understanding of the relative arrangement of the muscles of the tongue it is intended to give beforehand a full description of the stroma or connective tissue framework of the tongue as this not only separates the different muscles, but also serves as the site of attachment of these muscles.

The connective tissue framework of the tongue may be said to comprise the tunica propria of the mucous membrane, the submucous tissue on the undersurface of the tongue, and the fibrous septa which exist in the substance of the tongue. These latter are the median, paramedian, and lateral septa. The median septum is well known both anatomically and morphologically as the fibrous partition in the middle line of the tongue. The latter two septa which are both bilateral, are, as far as we are aware, described for the first time in this investigation.

The *median septum*, which is the fibrous membrane separating the two halves of the tongue, reaches its full development in the portion which intervenes between the two transverse muscles. As it is traced dorsally, it becomes greatly reduced in thickness and is very rarely seen to reach the mucous membrane of the dorsum of the tongue. In fact it is hardly possible, in most localities, to recognize microscopically any membranous septal formation between the two superior longitudinal muscles. Ventrally the septum is made up of a loose connective tissue which intervenes between the two genioglossus muscles (see Pl. I, figs. 3, 4, 5, 6, 9 and 10). Anteriorly it narrows dorso-ventrally and becomes continuous with the extremely thickened area of the lamina propria of the tip of the tongue which I have called the anterior arch. Posteriorly it narrows and flattens abruptly to become continuous with the hyoglossal membrane (see Text-fig. 7).

The *paramedian septum* is the thickest membranous structure in the substance of the tongue. It extends from the lowest part of the central portion of the median septum as far as the tunica propria of the mucous membrane on the lateral part of the ventral surface of the tongue. It applies itself to the outer surface of the genioglossus muscle, and in transverse sections of the tongue it can be seen that medialwards the septum separates the transverse and vertical muscles dorsally from the genioglossus muscle ventrally, while lateralwards it separates it from the inferior longitudinal muscle (see Pl. I,



Text-fig. 7. Drawing of diagram illustrating the membranous part of the tongue. A. Side view of the three septa of the tongue as seen from the left side. The divisions of the median septum and the hyoglossal membrane are seen. B. The three septa as seen in a frontal section. The anterior arch and the hyoglossal membrane are shown. C. A transverse section at the posterior third of the tongue illustrating the three septa and the two parts of the lateral septum.

figs. 4, 5 and 6). It is triangular in shape, the apex of the triangle lying anteriorly on the median septum. The base of the two septa continues posteriorly as the hyoglossal membrane. The hypoglossal nerve and the lingual vessels run in the substance of this septum as they proceed into the tongue (see Pl. I, fig. 6 and Text-fig. 7).

The *lateral septum*, which exists only in the posterior third of the tongue, is a fairly thick connective tissue membrane which lies between the inferior longitudinal muscle medially and hyoglossus and styloglossus muscle laterally. It has also the shape of a triangle whose apex ends on the paramedian septum

anteriorly, and whose base passes into the substance of the hyoglossal membrane posteriorly (see Pl. I, fig. 6 and Text-fig. 7).

Obviously the hyoglossal membrane, therefore, is formed anteriorly by the coalescence of the flattened portion of the paramedian septum, and the continuation of the bases of the paramedian and lateral septa of both sides of the tongue. Posteriorly this membrane becomes attached to the anterior part of the body of the hyoid bone (see Text-fig. 7).

The *anterior arch of the tongue.* It is worthy of note that in practically all the material examined, the lamina propria of the mucous membrane at the very tip of the tongue is condensed into a thick fibrous cord. This fibrous cord is placed parallel to the edge of the tip of the tongue and has an arched form—with its concavity directed backwards. Into this are inserted the muscular fasciculi of the most anterior fibres of superior longitudinal, genioglossus, and inferior longitudinal and styloglossus muscle dorso-ventrally. We propose to call this the anterior arch (see Pl. I, figs. 1 and 2 and Text-fig. 7).

Musculature of the tongue

The *superior longitudinal muscle* takes origin in conjunction with the corresponding muscle of the opposite side, from the antero-median part of the body of the hyoid bone, as well as from the hyoglossal membrane and the dorsal mucous membrane of the corresponding side. Its fibres proceed anteriorly towards the tip of the tongue to become inserted partially into the lamina propria of the dorsal mucous membrane which covers this muscle, and finally into the anterior thickened cord called the anterior arch. The muscle reaches its maximum thickness about the middle part of its length, and is usually triangular in cross section, possessing a dorsal, medial and ventro-lateral surface. Its dorsal surface lies directly under the dorsal surface of the mucous membrane. Its medial surface faces that of the corresponding muscle of the opposite side, while the ventro-lateral surface bounds the decussating fibres of the transversus and verticalis muscle (see Pl. I, figs. 4, 5). Anteriorly the muscle flattens dorso-ventrally to reach its termination (see Pl. I, fig. 3), while posteriorly and beyond its attachment to the hyoglossal membrane, it becomes oval in shape and just in front of the body of the hyoid bone it joins the corresponding part of the opposite side (see Pl. I, figs. 6, 7).

The *inferior longitudinal muscle* is a flattened band medio-laterally, separated in the anterior part of its posterior half from the genioglossus muscle by the para-median septum and from the styloglossus muscle by the lateral septum (see Text-fig. 1). As it is followed towards the root of the tongue, its fibres, after decussating with the fibres of the genioglossus muscle, are attached in conjunction with the latter muscle to the antero-lateral part of the body of the hyoid bone. A few of its most lateral fibres, however, were also seen in a tiger's tongue dissected by the author to originate in common with the hyoglossus muscle from the lesser cornu of the hyoid bone. Anteriorly, about the middle of the tongue the muscle blends with the styloglossus muscle,

the anterior fibres of hyoglossus, and genioglossus muscles, and then runs as one single oval fleshy mass on the ventro-lateral aspect of the tongue. In front of the anterior border of the genioglossus muscle this fleshy mass becomes gradually reduced in size, as a result of successive termination of these combined fibres in the lamina propria of the surrounding mucous membrane. More anteriorly this mass flattens transversely, approaches the median plane, and joins that of the opposite side, to become inserted into the anterior tendinous arch just ventral to the insertion of the superior longitudinal muscle (see Text-fig. 1).

Musculus transversus. It takes origin from the middle part of the median septum. Its most dorsal fibres take a dorso-lateral direction towards the lamina propria of the lateral part of the dorsal mucous membrane. The middle fibres run more or less transversely, while the ventral fibres take a ventro-lateral direction. The fibres may either reach the lamina propria, or stop short at the surrounding muscles and their septa (see Pl. I, figs. 3, 4 and 5).

The *musculus verticalis* extends ventro-dorsally in bundles or laminae which decussate with similar bundles or strata from the transverse muscle. Some of its fibres can be traced dorsally through the superior longitudinal muscle as well as ventrally through the inferior longitudinal and styloglossus muscles into the lamina propria of the dorsal and ventral surfaces respectively. The rest of the fibres do not reach the mucous membrane, and apparently they find attachment into the tongue septa or the sheaths of its muscles (see Pl. I, figs. 3, 4 and 5).

Both musculus transversus and verticalis reach their maximum thickness about the middle of the tongue. The bulk of both muscles diminishes anteriorly and posteriorly. Both muscles can be seen in sections through the anterior three quarters of the tongue. The extent of the musculus verticalis is shorter than musculus transversus (see the above text-figures).

The *genioglossus muscle*, which is the most extensive muscle in the tongue, can be seen in any transverse section through its substance. It extends through the whole length of the tongue parallel to its fellow of the opposite side, and separated from it by the ventral part of the median septum. The muscle arises from the posterior part of the symphysis menti. Its fibres radiate antero-posteriorly as well as medio-laterally in a fan-shaped manner in the substance of the tongue. Its most anterior fibres take a curved course towards their attachment into the anterior tendinous arch. Its most posterior fibres become attached partly to the hyoglossal membrane medially, and partly with the inferior longitudinal muscle into the antero-lateral part of the body of the hyoid bone laterally. The rest of its fibres are attached to the paramedian septum. Its medial fibres are more or less vertical, while the lateral ones diverge laterally and dorsally (see Pl. I, figs. 3, 4, 5, 6 and 7).

The *styloglossus muscle* arises from the lateral surface of the great cornua of the hyoid bone—or processus styloideus of the os hyoideus—near its posterior extremity. As it courses forwards in the substance of the tongue it passes

superficially to the hyoglossus muscle. Just before the middle of the tongue postero-anteriorly, it winds round the lateral and inferior parts of the inferior longitudinal muscle, where it is separated from it by the lateral septum. More anteriorly it blends with the latter muscle to have a common insertion into the anterior tendinous arch, as well as into the lamina propria of its locality (see Text-fig. 1, and Pl. I, figs. 5, 6 and 7).

The *hyoglossus muscle* is a small quadrangular muscle, running obliquely both dorsally and anteriorly, situated at the root and side of the tongue, between styloglossus muscle laterally, and inferior longitudinal and genioglossus muscle medially. It originates as a fleshy bulky muscle—in conjunction with a few of the most lateral fibres of the inferior longitudinal muscle—from the lesser cornua of the hyoid bone. As it is directed dorsally and anteriorly it flattens gradually into a thin sheet whose fibres are radiating postero-anteriorly in a fan-shaped manner, passes between the superior longitudinal muscle dorsally, and genioglossus ventrally, interdigitating with both and is inserted partly in common with the latter into the hyoglossal membrane and the paramedian septum, and partly with the inferior longitudinal muscle, in addition, to the anterior arch (see Text-fig. I, and Pl. I, figs. 7, 6, 5 and 4).

DISCUSSION

The division of the lingual muscles into extrinsic and intrinsic groups has led to the general belief that the extrinsic lingual muscles are those responsible for the different movements of the tongue, while the intrinsic ones are concerned with variations in its form. This is not the case as most, if not all, of the intrinsic muscles are involved in every movement of the tongue. Indeed in many movements both intrinsic and extrinsic muscles so called, are working together.

It is to be noted further that the greater portion of what we call the extrinsic muscles lies, in fact, inside the substance of the tongue. In a way these muscles can therefore be regarded as intrinsic. Apart from simplifying matters to the student of anatomy, the division of the lingual muscles into extrinsic and intrinsic groups is of no proper scientific significance.

The movements of the tongue occur as the resultant of contraction, either partially or wholly, of its different muscles, which muscles may act together or antagonistically. Owing to the intricate interlacement of these muscles, especially the intrinsic ones, it has been hitherto difficult to determine their individual action. In the above experiments we tried to find out the action of each muscle either by direct stimulation of its nerve supply, or by the way of exclusion.

It was previously thought that the contralateral deviation which occurs on protrusion of the tongue in cases of unilateral paralysis of the hypoglossal nerve is produced by the unopposed action of the genioglossus muscle. This was first disproved by Bennett who found that while stimulation of the trunk of the hypoglossal nerve protrudes the tongue and deviates its tip to the stimu-

lated side, stimulation of the terminal branch of this nerve deviates the tongue to the opposite side. The author concluded that the terminal branch is wholly distributed to the intrinsic lingual muscles, and that the latter muscles are therefore those concerned with the contralateral deviation of the tongue. We are in complete agreement with Bennett, because if the genioglossus muscle takes really any part in contralateral deviation of the tongue, one would have expected the tip of the tongue to deviate more to the opposite side on stimulation of the trunk of the hypoglossal nerve, than on stimulating its terminal branch. Contrary to this expectation we found, as did Bennett, that stimulation of the trunk, i.e. stimulation of the nerve twigs to genioglossus, as well as the terminal branch of Bennett, deviates the tongue to the same side. This result excludes every possibility of the genioglossus or any other extrinsic muscle participating in contralateral deviation of the tongue.

We have found that stimulation of the nerves to the genioglossus muscle causes a forward protrusion of the tongue with deviation of its tip towards the stimulated side. The old conception that the shifting of the tip of the tongue on protrusion in unilaterally paralysed patients is done by the unopposed action of the genioglossus muscle must be discarded.

As it is recorded in Exp. I and Text-fig. 2, stimulation of the trunk of the hypoglossal nerve results in protrusion and homolateral deviation of the tongue; its tip being rotated downwards. When this was repeated after exclusion of the intrinsic muscles—through division of their nerve supply—tongue protrusion was diminished while the homolateral deviation was markedly increased (Exp. IV). It is obvious therefore that the intrinsic muscles take an active part in protrusion of the tongue, and at the same time antagonize such homolateral deviators as the genioglossus muscle. If the intrinsic muscles were really antagonistic in action to homo-deviators, we should expect them to shift the tongue to the opposite side when they alone are thrown into contraction. This was found to be actually the case. For, as can be seen from Exp. VIII (Text-fig. 4), stimulation of the hypoglossal nerve, after exclusion of the extrinsic muscles, led to a contralateral deviation of the tongue. Besides, there was also protrusion, narrowing and flattening of the body of the organ, and a dorsiflexion of its tip. The narrowing and flattening are apparently produced by the action of the transversalis and verticalis muscles respectively. The contralateral deviation is undoubtedly performed through the action of the superior longitudinal muscle, because when as in Exp. XII the muscle was divided transversely, this type of deviation disappeared completely. The course, arrangement, and situation of the fibres of this muscle, and the fact that its longest fibres which constitute the main bulk of the muscle, occupy a medial situation to its shorter and less aggregated ones, are all in favour of such an action.

As can be seen from Exp. VIII and Text-fig. 4, one of the results of stimulation of the nerves to the intrinsic muscles—with the exception of the inferior longitudinal muscle—is an upward rotation, or as called above, a dorsiflexion

of the tip of the tongue. When on the other hand the superior longitudinal muscle is divided transversely so as to exclude its action, and the same nerves stimulated again, the tip of the tongue protrudes forwards without any rotation—either upwards or downwards (Exp. XII and Text-fig. 6). Besides being a contralateral deviator of the tongue, the superior longitudinal must also be the muscle concerned with upward rotation or dorsiflexion of the tip. It is clear that neither the transverse nor the vertical muscle has anything to do with dorsi- or ventroflexion of the tip. On comparison of the results of Exps. VII and VIII, it will be clearly concluded that the inferior longitudinal muscle aided by fibres of the styloglossus muscle and others is consequently concerned with downward rotation—ventroflexion—of the tip.

CONCLUSIONS

1. Neither in cases of unilateral paralysis, nor in normal cases has the genioglossus muscle anything to do with contralateral deviation of the tongue. The action of this muscle is to pull the tongue to the floor of the mouth, to protrude it, and deviate its tip to the same side.

2. The superior longitudinal muscle has been found to be the only contralateral deviator of the tongue as well as the only dorsiflexor of its tip.

3. The inferior longitudinal muscle—aided by the styloglossus muscle and anterior fibres of hyoglossus and genioglossus muscles—is, besides being a homo-deviator, a ventroflexor of the tip.

4. Both the superior and inferior longitudinal muscles possess a bony attachment to the hyoid bone.

5. In most animals the hypoglossal nerve appears to divide on the under surface of the tongue into medial and lateral division. Nerves to the extrinsic muscles and to the inferior longitudinal muscle arise either from the trunk of the nerve or from its lateral division. The medial division supplies the intrinsic muscles including the inferior longitudinal muscle. It sends also few twigs to the genioglossus muscle.

6. A description of the architecture of the tongue has been given.

I wish to express my best thanks to Prof. D. E. Derry under whose supervision this work has been done, and to Prof. G. V. Anrep in whose department the work has been carried out. The work was done at the suggestion of Dr A. A. Tarkhan, whom I wish to thank for his invaluable guidance and criticism. I am much indebted to Dr H. Kashif and A. M. Fargally for their kind help in doing the experiments.

ABBREVIATIONS

S.L.M.	Superior longitudinal muscle.	*M.S.*	Median septum.
I.L.M.	Inferior longitudinal muscle.	*D.M.S.*	Dorsal part of the median septum.
T.M.	Transverse muscle.	*M.M.S.*	Middle part of the median septum.
V.M.	Vertical muscle.	*V.M.S.*	Ventral part of the median septum.
G.G.M.	Genioglossus muscle.	*P.S.*	Paramedian septum.
H.G.M.	Hyoglossus muscle.	*L.S.*	Lateral septum.
S.G.M.	Styloglossus muscle.	*P.F.*	Pad of fat.
G.H.M.	Geniohyoid muscle.	*B.H.B.*	Body of the hyoid bone.
M.H.M.	Mylohyoid muscle.	*P.T.*	Palatine tonsil.
H.N.	Hypoglossal nerve trunk.	*D.*	Decussation.
M.H.N.	Medial division of hypoglossal nerve.	*M.G.*	Mucous glands.
L.H.N.	Lateral division of hypoglossal nerve.	*S.G.*	Serous glands.
L.A.	Lingual artery.	*S.G.L.*	Sublingual gland.
L.V.	Lingual vein.	*M.M.*	Masseter muscle.
A.A.	Anterior arch.	*H.M.*	Hyoglossal membrane.

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EXPLANATION OF PLATE I

Fig. 1. Microphotograph from a transverse section of the cat's tongue at its tip, showing the dense mass of the lamina propria of connective tissue (anterior arch), whose fibres are running in all directions.

Fig. 2. Microphotograph from a horizontal section at the tip of the tongue, showing the (anterior arch) giving attachment to the longitudinal muscle fibres which are inserted into the tip.

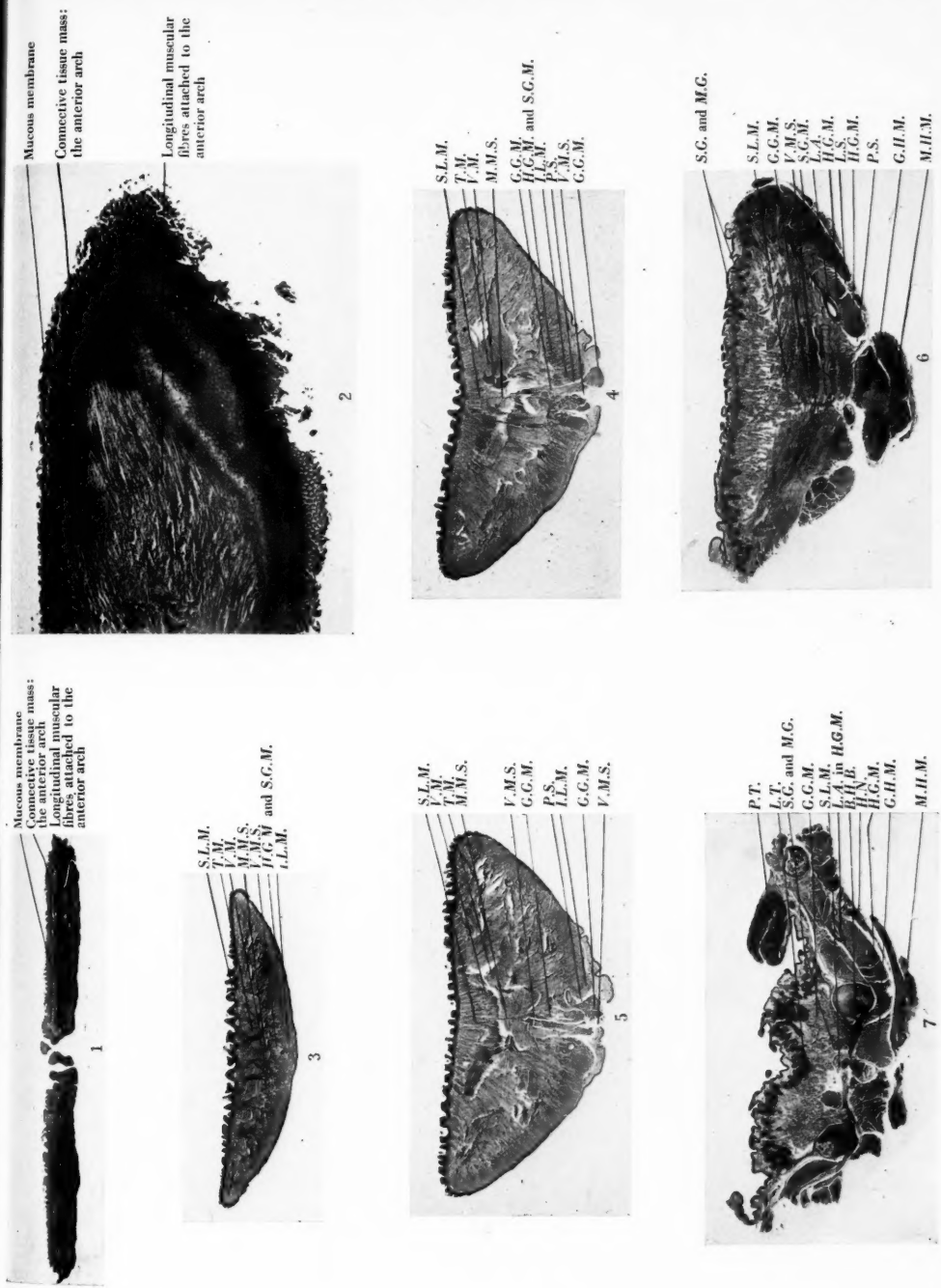
Fig. 3. Microphotograph from a transverse section at the anterior fourth of the tongue. The central part shows transverse and vertical muscle fibres mingled together and attached to the middle part of the median septum. These two muscles lie between sheets of muscular fibres, i.e. the superior longitudinal muscle dorsally, and genioglossus, hyoglossus, inferior longitudinal, and styloglossus muscles; forming one continuous mass of longitudinal fibres on the ventral aspect.

Fig. 4. Microphotograph from a transverse section at the anterior third of the tongue. Transverse and vertical muscles are growing bigger and extending more both dorsally and ventrally between the fasciculi of the surrounding muscles. The superior longitudinal muscle assumes a triangular form. The genioglossus muscle can be seen with the paramedian septum—with the lingual artery in its substance—separating it from the inferior longitudinal, hyoglossus, and styloglossus muscles. Both the middle and ventral parts of the median septum can be made out.

Fig. 5. Microphotograph from a transverse section at the middle of the tongue. The superior longitudinal muscle appears triangular. The transverse and vertical muscles are largely increased at the expense of more or less vertical fibres derived from genioglossus muscle, which cannot be differentiated from the vertical muscle and which intermingle with the strata of the transverse muscle. A good number reach the dorsal mucous membrane of the tongue. The inferior longitudinal muscle is separated by the paramedian septum from the genioglossus muscle medially, and indistinctly localized laterally from the adjacent muscles.

Fig. 6. Microphotograph from a transverse section at the posterior third of the tongue. The superior longitudinal muscle is decreasing especially laterally as the genioglossus muscle increases to practically more than half of the section. The former muscle is largely interrupted by the interdigitating fibres of the genioglossus whose medial and ventral fibres decussate with their fellows of the opposite side. The three septa with their contents are seen. The hyoglossus which occupies a considerable part on the lateral and dorsal angle of the tongue, is partially identified from the styloglossus muscle. A pad of fat is seen in the substance of the posterior end of the ventral part of the median septum.

Fig. 7. Microphotograph from a transverse section at the level of the body of the hyoid bone, to the anterior and superior part of which, some of the fibres of the superior longitudinal muscle are attached in conjunction with some fibres of the genioglossus muscle. Inferior longitudinal with hyoglossus muscle are attached to the lateral part of the body of the same bone. Hyoglossal membrane is also seen joined to the latter bone. Hyoglossal muscle is separated from the styloglossus muscle by a fairly wide interval. Palatine tonsil and cross-section of the middle cornua of the hyoid bone are seen in the most lateral angle of the section.


Mucous membrane
Connective tissue mass: the anterior arch
Longitudinal muscular fibres attached to the anterior arch
1
2
3
4
5
6
7
S.L.M.
T.M.
V.M.
M.M.S.
V.M.S.
H.G.M. and S.G.M.
I.L.M.
G.G.M.
P.S.
G.H.M.
M.H.M.
S.G. and M.G.
P.T.
I.T.
L.A. in H.G.M.
B.H.B.

UNIV. OF MICH.

PLATYMERIA AND PLATYCNEMIA

By L. H. DUDLEY BUXTON
Department of Human Anatomy, Oxford University

It is hardly necessary to describe at length the accepted views about platymeria and platycnemia. Briefly, it has been suggested that this flattening of the two large bones of the lower extremity is due to either posture or gait or both, or to such activities as mountain climbing. It has been suggested that it may be associated with the relative development of certain muscles which are attached to the femur and tibia.[1] In one of the most recent discussions on the matter Cameron[2] maintains that platymeria cannot be due to squatting because it is absent in the Eskimo who squat. He suggests that it is due to unwonted strain during childhood and early adolescence. Platycnemia, he maintains, is not necessarily associated with platymeria but is due to squatting and is the result of undue prominence of the origin of m. tibialis posticus. Bilateral bowing of the humerus in Minorcans he attributes to the unusual muscular exercise associated with slinging.

It is generally accepted that platymeria and platycnemia occur more frequently among modern primitive peoples and early man in Europe than among peoples in a more advanced state of culture, and that the condition cuts across the main systematic racial divisions of mankind.

During an examination of a series of ancient bones from Kish in Mesopotamia, I came to the conclusion that the accepted views were not altogether satisfactory.[3] I have since collected further material apposite to the problem.

A preliminary examination of the available material suggested that a fruitful line of enquiry would be an investigation into the flattening, within groups, and the relation of the absolute measurements was first tabulated. Next the association of the various indices with one another has been studied, first within groups, on a single series of sixty Romano-British skeletons, the longest at my disposal, i.e. intraclass correlation, and secondly between groups, i.e. interclass correlation. Comparisons have also been made to show the effect of age, side and sex.

Cameron[4] says that the platymeric index is, in Minorca, progressively lower with age. He bases his statement on three specimens and admits that a femur from Windmill Hill (Neolithic) is exceptional. Cameron's figures are, however,

[1] F. G. Parsons, vol. XLVIII, 1913–14, p. 259.

[2] John Cameron, *The Skeleton of British Neolithic Man*, 1934. See also the same author's chapter in *Cambridge Excavations in Minorca, Sa Torreta*, by Margaret A. Murray, 1934. But see Hrdlicka's figures, quoted below.

[3] *Journal of the Royal Anthropological Institute* (1931), vol. LXI.

[4] *Loc. cit.* p. 163.

too few for any inference to be drawn from them. Badouin,[1] on the other hand, who gives no measurements and no indication of the number of specimens on which his statements are based, takes exactly the opposite view, and says that both the humerus and femur (subtrochanteric region) in Neolithic children are flatter, that is, have lower indices, than adults, the children having a platymeric index from 75 to 90 and the adults from 90 to 100. The few bones of children which I have examined so far suggest that they are just as variable in their indices as adults.

The relation of flattening to side is an interesting point, and may be studied in two ways; the correlation between the indices on the same skeletons may be computed, or a difference taken between averages when it is not possible to pair the bones. The correlation between sides is quite high, especially on the femur and humerus on my longest series of skeletons (Romano-British males), as can be seen from the table.

Correlation between sides, indices of flattening

Femur	Tibia	Humerus	Radius
+0·7413 (55)	+0·5298 (48)	+0·6722 (46)	+0·6148 (35)

Numbers in brackets give the numbers of pairs on which the correlations are based.

High correlation does not necessarily imply the same mean values, but in the few series in which I have been able to make accurate tests there is no significant difference between the sides except for the Romano-British humeri, which are markedly more flattened on the right than the left side.

Data for comparison between the sexes are even more scanty. Pearson in his long series of English thigh bones concludes that on both sides there is a significant difference.[2] My own data are not sufficient, but at least there are enough to say that the habit which some authors have of forming a "group" average based on combined male and females bones is an unjustifiable procedure.

Turning next to the relations between bones, I have only been able to make a single series of intraclass correlations, that is, comparisons on a series of skeletons belonging to the same group. An examination of the Romano-British male skeletons showed that, within a single group, except for the correlation between the flattening of the subtrochanteric region of the femur and the tibia in the level of the nutrient foramen, there was no correlation between the various bones which could not be attributed to random sampling. But even on this evidence it seems a justifiable conclusion that we must definitely suggest a hypothesis which will include femur and tibia together in our explanation of the causes of flattening.

[1] *C.R. Acad. Sci., Paris* (1918), vol. CLXVIII, pp. 129–31.

[2] *A Study of the Long Bones of the English Skeleton*, by Karl Pearson, F.R.S. and Julia Bell. Part I. *The Femur*, p. 142.

Correlation between flattening indices of bones of the same skeleton

	Femur-tibia	Femur-humerus	Tibia-humerus	Tibia-radius	Humerus-radius
Left side	+0·3579 (52)	−0·1759 (50)	+0·1611 (44)	+0·0105 (37)	−0·1303 (34)
Right side	+0·4441 (54)	−0·0639 (49)	+0·1560 (46)	−0·1835 (30)	+0·1415 (38)

I have given all the correlations, since they are at least consistent, but owing to the fact that the radii are often broken, numbers are too few. Using Fisher's z transformation, we find that there is no significant difference between the correlations on the right and left side between femur and tibia, and that apart from the correlation between the flattening of the femur and tibia the other correlations are insignificant.

For correlation between groups owing to paucity or different methods of measurement, it is only possible to test the relationship between the humerus and the femur. The correlation between the platymeria and platybrachia should be noted[1] (see Table I).

Table I. *Table showing indices of flattening (groups)*

	Left		Right	
	Femur	Humerus	Femur	Humerus
United States (Whites), Modern	84·1 (66)	84·1 (?)	82·8 (66)	81·9 (?)
Modern Maltese	83·9 (28)	78·5 (17)	84·9 (23)	78·0 (23)
Middle Ages: Oslo	82·9 (264)	80·3 (150)	83·0 (261)	78·8 (161)
Eighteenth-century Maltese	82·7 (104)	80·7 (50)	82·9 (93)	80·6 (47)
Modern N. China	80·7 (21)	78·5 (19)	78·9 (20)	75·7 (20)
Romano-Britons	79·2 (62)	82·2 (54)	80·4 (61)	79·1 (52)
Kish (Sumerian)	78·6 (14)	76·4 (12)	80·2 (12)	75·5 (13)
American Indians, Arkansas	76·6 (15)	75·4 (10)	76·7 (15)	74·4 (10)
Louisiana	74·6 (23)	77·8 (18)	73·2 (23)	76·8 (18)
Munsee	71·6 (15)	74·6 (13)	74·3 (15)	73·6 (14)

As the number of groups is so small this is best indicated by a diagram,[2] which shows graphically how, if we take racial means, low indices on the humerus are always associated with a flattening of the femur in the subtrochanteric region. The lengths of the upright and horizontal strokes marking the position of each group correspond to the square root of the number of

[1] The platybrachic index may be defined as the percentage ratio of the minimum to the maximum diameter of the diaphysis of the humerus measured in the centre of long axis of the diaphysis.

[2] I owe the form of this diagram to the suggestion of Prof. E. S. Pearson.

cases used to find the mean value of the platymeric and platybrachic index respectively in each case and indicates the reliability of the figures. Actually the interracial correlation coefficients are +0·7405 and +0·7991 for right and left bones respectively.

This evidence suggests the possibility of two sets of factors in the production of platymeria and platycnemia. Within a single group (the Romano-British series) there is a definite association of flattening of the femur and tibia, but a complete dissociation of flattening of the humerus and radius. Moreover, the distribution of individual indices varies over nearly the whole range found in mankind—for it is to be noticed that the mean value of the Romano-British indices is close to the mean for all mankind. As the group is probably fairly

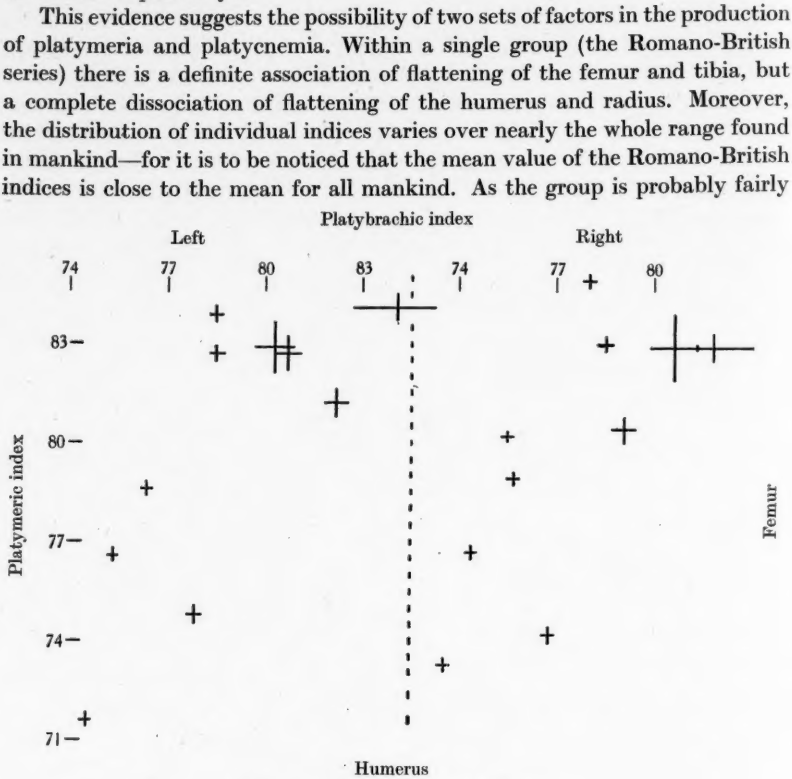


Diagram 1. For explanation see text.

homogeneous, mechanical factors as, for example, squatting, should be common to the whole group.

If instead of individuals the means of groups are considered, there appears to be some factor at work which affects all the limbs; this factor must be of a general nature, for it can hardly be posture or gait, which would affect the lower limb only. It is noticeable that, on the whole, flattening decreases with advancing civilization, the only exceptions apart from a very short Nigerian one is a long Eskimo series. Alternatively the increased flattening may be due to physiological rather than mechanical causes, or possibly a combination of these two factors may be at work. It can readily be shown that, assuming the area for the muscles is kept practically constant, less actual bone substance

is required in the construction of the shaft of a flattened bone than of a cylindrical one. This can be subjected to direct observation. Ten femora were measured and a transverse section made in the subtrochanteric region. The total area of this section was measured with a planimeter. It was found that if the section of the femur was treated as an ellipse the computed area agreed reasonably closely with the area observed by planimeter. If we can treat the shape of the subtrochanteric region as that of an ellipse for a given perimeter, the more closely the two semiaxes approach one another in length the greater the area.

The area, perimeter and platymeric indices were then calculated for such data as were comparable. It will be seen from Table II that, apart from a few exceptions, the perimeter is very constant; out of thirteen examples no less than eight are between 9·1 and 9·4, where the platymeric index in the same femora varies from 72·0 to 85·3. The Romano-British series have an exceptionally large perimeter and area, and the Yukon Indians and the Kish material, both small samples, have a small area and perimeter. The figures suggest that the platymeric index and perimeter are independent. (See Table II.)

Table II. *Male platymeric index in relation to area and perimeter*

(Left bones, except * which are pooled left and right.)

Group	No.	Index (platymeric)	Area index†	Area cm.²	Perimeter
Yukon Indians*	14	70·7	67·28	5·86	8·71
Munsee Indians	15	72·0	67·03	6·22	9·28
Pecos Pueblo	157	73·9	67·33	5·83	8·66
S.W. Eskimo*	195	75·0	71·85	6·61	9·20
Cyprus, Neolithic	10	75·3	73·36	6·19	8·97
Malta, Neolithic	8	76·0	70·83	6·58	9·29
Kish (Sumeria)	13	76·8	67·77	5·87	8·66
Igloo Eskimo*	33	77·2	76·54	7·47	9·76
W. Eskimo*	100	78·1	72·63	6·74	9·28
Romano-Britons	46	81·8	80·04	8·10	10·12
Malta, eighteenth century	104	82·7	73·83	6·91	9·36
Oslo, Mediaeval	261	82·9	74·74	7·12	9·50
Malta, Modern	28	83·9	72·87	6·72	9·23
U.S., Modern	66	84·3	73·88	6·93	9·38
London plague pits	247	85·3	72·10	6·59	9·14

† $\frac{\text{Area} \times 100}{\text{Perimeter}}$.

We have then a cylinder whose surface, as judged from a section, tends to be fairly constant, but this section tends to have a very variable area. In other words we get a column which tends to be flattened when there is a shortage of bony material and rounded when there is an abundant supply, though the figures suggest that there may also be other factors at work.

The cause of this bone deficiency is a difficult point; it may be due to a calcium deficiency or a vitamin deficiency, but nutritional factors are at present so little known that no definite theory can be put forward. It is

suggested that this is a line well worthy of enquiry. The individual differences which occur may reasonably be explained by this method since, whereas posture and gait are common to a group, individual tastes in food and individual reactions to the same food vary considerably.

SUMMARY

It is suggested that the accepted views on the causation of platymeria and platycnemia do not account for the flattening which also occurs in the bones of the arm. An investigation showed that the correlation between the flattening of the femur and humerus was too high to be fortuitous. Low indices of both bones are found among primitive peoples and high among civilized. It is suggested that this flattening is due to a deficiency of bone, in relation to the area needed for muscular attachments. Possibly the diet of primitive peoples may help to explain this condition.

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THE THALAMUS OF THE CHIMPANZEE

IV. THALAMIC PROJECTIONS TO THE CEREBRAL CORTEX[1]

A. EARL WALKER, M.D.[2]
Division of Neurology and Neurosurgery, University of Chicago

From the Laboratory of Physiology, Yale University School of Medicine, New Haven

I. INTRODUCTION

THE pioneers in the study of cerebral architecture considered the peculiarities of the minute structural arrangement of the cerebral cortex to indicate its functional activity and hence to be related to its afferent and efferent connexions. The thalamus, then, as the greatest subcortical contributor of afferent fibres, was considered to play a significant role in the mapping of the cerebral cortex (Smith, 1910). It is completely in accord with this conception that recent studies have demonstrated the close correlation between the cortical projection areas of the individual thalamic nuclei and the cytoarchitectural fields of the cerebral cortex. During the past five years these thalamocortical relationships have been largely determined for the monkey (*M. mulatta*). Man, however, is considerably removed in the primate scale from the monkey, and it is not yet clear how far these relationships may be applied to the human being. For this reason, the investigation of the thalamic projection to the cerebral cortex of an anthropoid such as the chimpanzee becomes increasingly pertinent and valuable.

The delineation of the cytoarchitectural and myeloarchitectural areas in the cerebral cortex of the chimpanzee has not yet been completely accomplished. Campbell (1905) in his epoch-making studies on the localization of cerebral function mapped the general cytoarchitectural fields of this animal. Six years later Mauss (1911) plotted the divisions of the chimpanzee cortex on the basis of the myeloarchitecture. The following account of the cortical architectural areas, and the cytoarchitectural map presented, are largely based upon these pioneer studies, some recent, more detailed investigations of individual parts of the cerebral cortex (Beck (1928) on the sylvian region, Bucy (1935), Strasburger (1937) and Walker & Green (1938) on the frontal lobe) and

[1] This work was aided by grants from the Research Funds of the Yale University School of Medicine and by a special grant for the purchase of chimpanzees from the Rockefeller Foundation.

[2] This research was carried out while the author was a Fellow of the Rockefeller Foundation in the Laboratory of Physiology, Yale University School of Medicine.

numerous observations made in the course of the present research. The nomenclature used by Brodmann (1909) and Vogt (1919) has been adopted.

Considerable investigation is necessary to clear up the remaining problems in cerebral architecture of the chimpanzee. The cytoarchitectural fields of the prefrontal region of this animal (areas 9, 10, 11 and 12) have not yet been delineated. The details of the cytoarchitectural divisions of the temporal and occipital lobes are little better known. The divisions and even the nomenclature of the architectural fields of the parietal lobe are confused. The cortex of the superior and inferior parietal lobules consist of distinct cytoarchitectural types. Brodmann (1909) has described the cortex of the inferior parietal lobule of the macaque as area 7, but in man the area so designated is placed above the intraparietal fissure. Although histologically these areas may appear to be so differently placed in monkey and man, because there seems to be a direct homology between the intraparietal sulcus of monkey, ape and man, the aptness of this terminology may be questioned. Foerster (1935), on the basis of the responses to electrical excitation in man, has designated the inferior parietal lobule as area 7, and the superior lobule as area 5 in accordance with the divisions in the monkey. This nomenclature has been followed in the present study, for it appears likely that the cortex above the intraparietal sulcus is an elaboration of area 5 type of cortex. For that reason the cortex of the superior parietal lobule and inferior parietal lobule will be considered as areas 5 and 7 respectively, it being understood that the two are only roughly comparable to similarly designated areas in lower primates.

With this brief discussion of the cerebral cytoarchitectural areas, a knowledge of which is essential to the understanding of the cortical projection of the thalamic nuclei, the efferent connexions of the thalamus will be considered.

It is now widely recognized that only certain of the thalamic nuclei project to the cerebral cortex. This is made evident by examination of the thalamus of a chimpanzee from which one hemisphere of the cerebrum has been entirely removed (Walker, 1938*b*). In such a preparation the nuclei of the midline, intralaminar nuclei (nuclei paracentralis, parafascicularis, limitans, centralis lateralis and centrum medianum) and the rostral tip of the lateral nuclear mass of the thalamus appear entirely normal. The remaining thalamic nuclei are almost completely degenerated. With slight reservations, therefore, it may be stated that the latter nuclei send their fibres to the cerebral cortex. Study of the hemidecorticate primates give no indication of the character of the topical thalamic projections. Only by studying the cellular degenerations in the thalamus following smaller cortical lesions can this be determined.

Material and technique

Animals. The animals used in this investigation were a group of sexually immature chimpanzees (*Pan satyrus*), aged 3–9 years, which had been under observation for at least a month preceding the primary operation. Some were

sufficiently tame to permit detailed neurological examination before and after operation; others were so wild that such a procedure was inadvisable.

The majority of these animals have been used for numerous other studies. The chimpanzees "Mussai" and "Yama" (Exps. 1 and 2) had been studied for several years by Dr Fulton for experiments on the effects of lesions of motor and premotor areas. The chimpanzee "Lucy" (Exp. 3) had been trained by Dr Carlyle Jacobsen and intensively studied psychobiologically for several years before sacrifice (see Jacobsen, 1935). Chimpanzee "Maggie" (Exp. 6) had been trained to discriminate weight, texture and form by Dr T. C. Ruch, and then re-examined following operation for deficits in these modalities (Ruch *et al.* 1938). I am deeply grateful to these investigators for the privilege of examining the brains of their several valuable animals. Chimpanzee "Tommy" (Exp. 4) had been studied in conjunction with Dr Ruch, who trained the animal in texture discrimination and re-examined it after each operation. The remaining animals have in most instances been used for terminal stimulation experiments (Walker & Green, 1938; Denny-Brown, 1938; Hoff & Green, 1936).

Surgical procedures. All operations upon the brain were carried out under general anaesthesia aseptically in the Laboratory of Physiology, Yale University. An osteoplastic flap was reflected, the dura opened and the cortex mapped. The exact site, extent and position of the lesion could thus be determined. After making the lesion, the dura was firmly reapproximated, the bone flap replaced and the skin sutured. At the time of sacrifice, the animal was anaesthetized with ether, the bone flap elevated and the cortex stimulated. The vascular system was then perfused with normal saline followed in most instances by 95 % alcohol, although formalin was used in some cases.

Histological controls. After fixation the hemispheres were photographed and the gross lesions described in detail. The thalami were removed by blunt dissections, and embedded in celloidin. The lesions with a generous amount of normal cortex surrounding them were similarly treated. In all but two cases, in which only representative sections were taken, the entire lesion was sectioned serially at 25μ. The advantage of this control is obvious, for deep softenings and other vascular effects, if undetected, may give rise to erroneous conclusions. The thalami were also sectioned serially, and every 3rd or 5th section stained and mounted. Every 25th section of the cerebral cortex was stained and mounted so that a section was examined every 0·75 mm. throughout the lesion. By this means it was possible to localize accurately a lesion and determine its cytoarchitectural boundaries.

Illustrations. Numerous illustrations have been used to replace lengthy narrative, for they present a clearer, more precise and objective picture to the reader. The illustrations of the hemispheres showing the site and extent of the lesions are pen-and-ink copies of photographs of the brain. The drawings of the sections of the cerebral cortex and of the thalamus were made with the aid of an enlarger and the details filled in by microscopic control. The number under the drawing of each section indicates the section from which the sketch

was made. The lesions are marked in black, the degenerations are indicated by stippling.

Nomenclature. The terminology used in the descriptions of the configuration of the cerebral cortex follows the standard nomenclature employed in human descriptive neuroanatomy as adapted for the brain of the chimpanzee by Mingazzini (1913) and Walker & Fulton (1936). The thalamic divisions or nuclei of the chimpanzee have been discussed in a previous section. Their ideal classification would be based upon cytoarchitecture, myeloarchitecture and physiological considerations. At the present time the last is largely determined by the fibre connexions of the parts of the thalamus. It is advisable, then, to consider those parts of the lateral nuclear mass which have distinct afferent and efferent connexions as nuclei, especially if their cytoarchitectural and myeloarchitectural characteristics are compatible with such divisions. In the thalamus of monkey, chimpanzee and man large well-staining cells characterize the ventral moiety of the posterior half of the lateral nuclear mass (that part of the thalamus lying between the external and internal medullary laminae). It is this part of the thalamus which receives the fibres of the lemnisci and spinothalamic tracts and projects to the postcentral convolution. It has also a distinctive myeloarchitectural appearance. A narrow fibre lamella divides it into a medial portion, which borders the nucleus centrum medianum, and on account of its shape has been known as the "arcuate" nucleus, and a larger lateral portion. Following the terminology of the University of Michigan school of anatomists this entire ventral moiety has been called the nucleus ventralis posterior, its medial part the nucleus ventralis posteromedialis, and its lateral portion the nucleus ventralis posterolateralis. Anterior to this large nucleus is a nuclear group which is composed of slightly smaller, evenly arranged cells, whose cytoarchitectural pattern is determined by the presence or absence of transverse fibre tracts passing to the internal capsule. This portion of the thalamus extends dorsally and posteriorly above the nucleus ventralis posterior for a short distance and anteriorly reaches almost to the rostral extremity of the thalamus. Its posterior and dorsal boundary is readily recognized because the cells of the posterior half of the dorsal part of the lateral nuclear mass are more loosely arranged. The division is clear in sagittally cut sections of the thalamus. This large anterior division of the lateral nuclear mass receives the fibres of the brachium conjunctivum and projects to the motor and premotor areas. It has been termed the nucleus ventralis lateralis. At the extreme rostral extremity of the lateral nuclear mass are clumps of cells which do not undergo degeneration even when the entire cortex is removed. These have been termed the nucleus ventralis anterior. The dorsal third of the posterior half of the lateral nuclear mass which does not receive fibres from the lemnisci nor the spinothalamic tract, and which projects to the parietal lobules, is logically distinguished from its neighbours and has been termed the nucleus lateralis posterior. This classification, based upon the functional relationship as well as the morphology of the thalamic parts, would appear to be a rational division of the thalamic nuclei.

II. THE TOPICAL CORTICAL PROJECTION OF THE THALAMUS

(1) Frontal lobe

Although neuro-anatomists have recognized an extensive fibre connexion from the thalamus to the cortex of the frontal lobe, neurologists have been slow to accept it. Their hesitation is based largely upon the premise that since the thalamus is considered a sensory ganglion its projections and projection areas should also be sensory. Clinically, however, no definite sensory disturbances have been demonstrated following lesions of the frontal lobe. For the same reason many have been loath to accept a thalamic projection to the precentral areas. It is now clear that the very foundations of this argument are unsound, and the anatomical facts, quite compatible with clinical data, are the basis for quite a different mechanism.

Recent experimental studies have demonstrated more and more emphatically that the frontal lobe is composed of two large functional divisions. Experiments both by ablation and stimulation have shown that the caudal half of the frontal lobe subserves predominantly a motor function, while the rostral moiety is concerned with integrations of a higher order. Correlated with these physiological characteristics are the differences in cytoarchitectural structure of those two parts of the frontal lobe. The cortex of the posterior portion is agranular, that of the anterior moiety, the association areas, is granular cortex. It would not be unexpected, then, to find that the thalamic projection to these two portions was quite distinct. Two experiments are presented which illustrate this difference.

Exp. 1. *Successive ablations of entire right and left frontal lobes, except for two leg areas; severe bilateral degeneration of the nuclei ventralis lateralis and medialis dorsalis, slightly more pronounced on the left side. Weight* 23·0 *kg.* "*Mussai.*"

The animal was a male chimpanzee which matured sexually during the period of observation; it had five successive lesions as follows: 29 April 1932, ablation of left arm motor area (4*b*); 13 May 1932, right arm area (4*b*); 11 November 1933, right frontal areas; 15 December 1933, left frontal areas; and finally 16 November 1935, section of the right cerebellar peduncles, following which the animal died in a few hours. Autopsy examination was made immediately. Until the final procedure the animal was in excellent physical condition.

Anatomical examination

The right cerebral hemisphere. The surface of the occipital, temporal and posterior part of the parietal lobes appear entirely normal.

The greater part of the cortex of the frontal lobe is ablated. The denuded area is covered with a shaggy arachnoidal membrane. A block of cortex is preserved in the superior part of the precentral convolution extending anteriorly 1·8 cm. along the midline. On the medial surface the margin extends obliquely downward to the rostrum of the corpus callosum. On the convexity it reaches to the depth of the central sulcus 1·8 cm. from the midline. Its lower margin follows the inferior frontal

to the central sulcus 1·7 cm. above the sylvian fissure. The tip of the frontal lobe lies about 2 cm. anterior to the inferior extremity of the central sulcus and is covered by a shaggy, ragged membrane. Through this can be seen the dilated anterior horn of the lateral ventricle. The cortex of the orbital surface of the right frontal lobe has been ablated except for a strip extending 1·9 cm. anterior to the optic chiasma. Although the postcentral sulci are widened and the postcentral convolution broadened, its cortex appears normal except for a few superficial abrasions along the posterior lip of the central sulcus (Fig. 2).

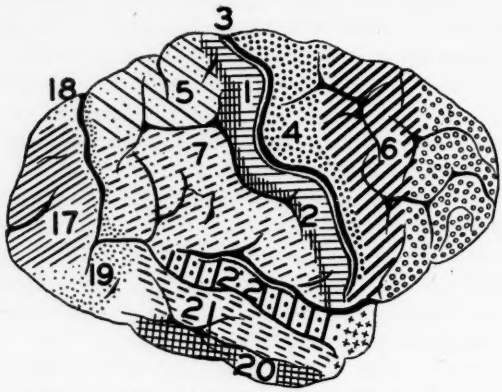

Fig. 1. Cytoarchitectural map of the cerebral cortex of the chimpanzee based upon observations in the present investigation and previous studies. The division of the prefrontal region (indicated by circles) has not yet been determined for the chimpanzee.

Examination of serial sections of the right frontal lobe confirms the gross location and extent of the ablation. The superior part of the precentral convolution composed of cortex characteristic of area 4 (Brodmann) is quite normal. In this area there is no premotor cortex (area 6, Brodmann) except for a thin margin anteriorly along the callosomarginal sulcus. Except for an occasional fragment of area 4 there is no motor or premotor cortex anterior to the middle third of the central sulcus. The lower part of area 4, which extends anteriorly to the posterior lip of the sulcus subcentralis anterior as well as the contiguous portion of area 6 remains intact. Most of the cortex on the superior lip of the inferior frontal sulcus is ablated, but area 6 and prefrontal cortex remain on the inferior lip. The cortex of the orbital surface of the frontal lobe is intact up to the line of ablation. On the medial surface the lesion begins a short distance anterior to the central sulcus and reaches the callosomarginal sulcus about 1 cm. posterior to the rostrum of the corpus callosum. Just behind the rostrum the ablation involves the cortex of the cingular gyrus and slightly anterior to it has removed the remainder of the frontal lobe. An irregular lesion damaging the cortex of the posterior lip of the central sulcus near its middle is the only significant lesion in the parietal lobe (Fig. 2).

Right thalamus. A careful examination of serial sections of the right thalamus shows very severe cellular degeneration in the nuclei medialis dorsalis and ventralis lateralis. That in the main medial nucleus begins near its rostral extremity and extends throughout its length. Almost all of the nucleus is involved in its posterior half, with only a ring of normal cells present about the margins. At about the middle of the thalamus (anteroposteriorly) scattered normal cells are present. A few sections anteriorly the degeneration becomes confined to the centre of the nucleus. The

degeneration in this position may be followed to the anterior extremity of the nucleus. In the lateral nuclear mass the degeneration begins caudally in the nucleus ventralis lateralis, which lies just above the nucleus ventralis posterior near the rostral extremity of the nucleus centrum medianum. It rapidly enlarges, and anterior to the nucleus ventralis posterior, which appears to be normal, the degeneration involves the entire lateral mass. However, in it are numerous groups of normal cells. In the

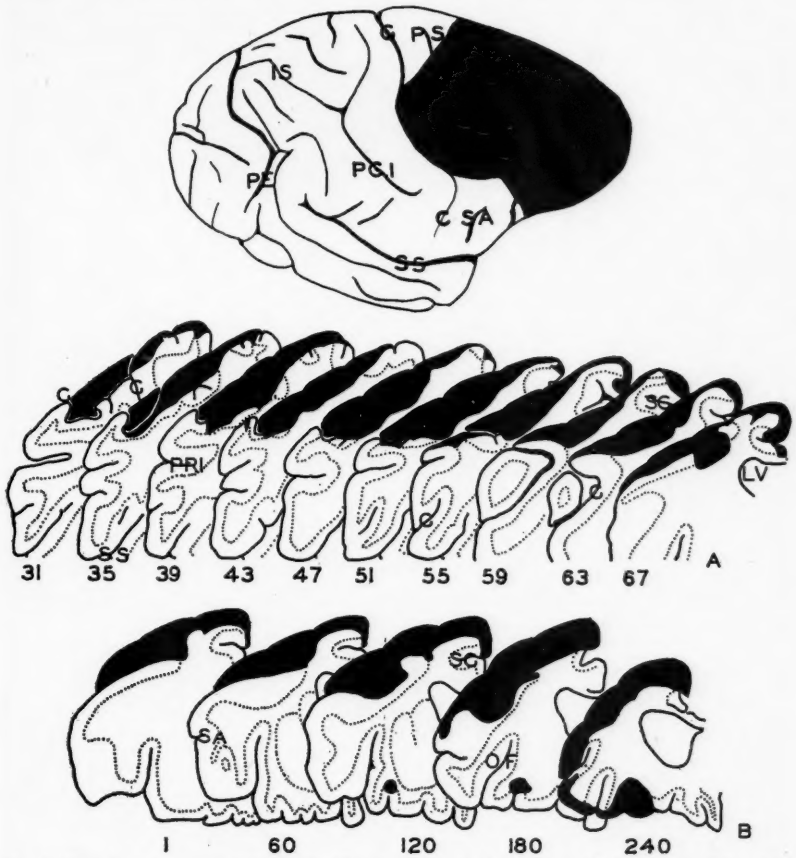

Fig. 2 (Exp. 1, chimpanzee "Mussai"). The site and extent of the cortical ablation of the right cerebral hemisphere. The serial sections of the lesion (below) are cut coronally, the most posterior section being in the upper left corner, and the most anterior in the lower right.

anterior parts of the nucleus the degeneration is severe, but a number of normal cells remain. In most of the anterior sections the latter occupy a zone about the middle of the nucleus parallel to the internal medullary lamina. There is an intense gliosis about the margin of the anterior ventral nucleus, but it probably represents fibre degeneration, rather than cellular alterations. This gliosis may be traced to the rostral extremity of the nucleus ventralis lateralis where normal cells remain. There is no degeneration in the nucleus anteroventralis or anterodorsalis (Fig. 3).

The left cerebral hemisphere. With the exception of a small abrasion of the inferior part of the postcentral convolution which appears to be a post-mortem artefact, since there is no reaction about it, the parietal, occipital and temporal lobes appear

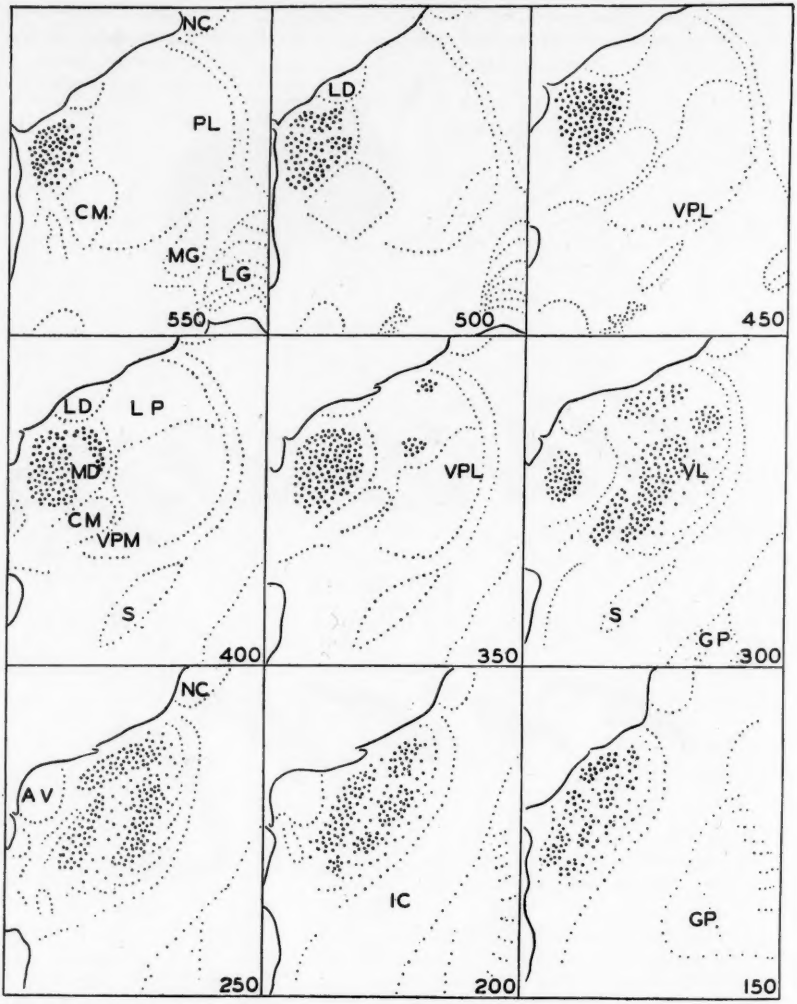

Fig. 3 (Exp. 1, chimpanzee "Mussai"). Representative serial sections of the thalamus showing the site and extent of the retrograde cell degeneration.

quite normal. The sulci of the anterior portion of the parietal lobe, especially the postcentral, are slightly widened (Fig. 4).

The cortex of the lateral surface of the left frontal lobe has been largely ablated. In the superior portion of the precentral gyrus is a block of cortex extending 2·2 cm.

anterior to the central sulcus along the midline and inferiorly for 1·8 cm. Along its middle third is a thin margin of the anterior lip of the central sulcus. Because of superficial lacerations it is difficult to determine the exact extent of the cortical damage. A strip of cortex posteriorly about 1 cm. in width and tapering anteriorly extends along the orbital margin to the frontal tip. The cortex above the superior lip of the callosomarginal sulcus and anterior to the rostrum of the corpus callosum

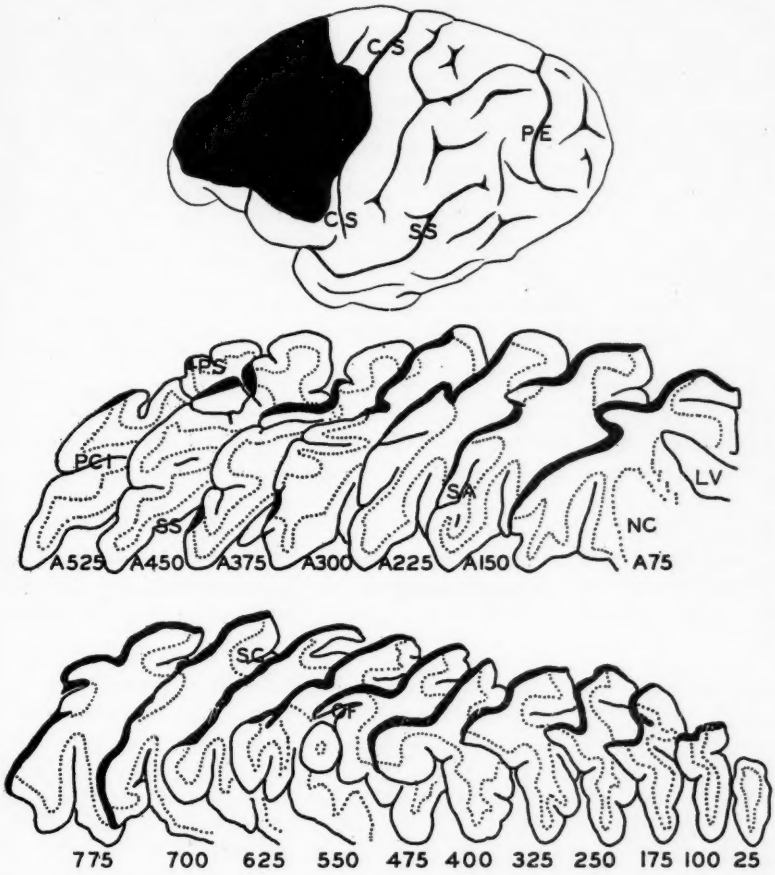

Fig. 4 (Exp. 1, chimpanzee "Mussai"). The site and extent of the cortical ablation of the left cerebral hemisphere. The serial sections of the lesion (below) are cut coronally.

on the medial surface of the hemisphere has been ablated. The margin follows the superior lip of the callosomarginal sulcus almost to the tip of the frontal pole. The subcallosal cortex on the medial surface of the frontal lobe, and that on the orbital surface of the frontal lobe, are uninjured.

An examination of the serial sections of the entire left frontal lobe confirms the gross location and extent of the ablation. The remaining precentral cortex along

the midline consists of a small zone of area 6 on the upper lip of the callosomarginal sulcus, and a block of area 4 near the midline. A small island of cortex in the mid-portion of the precentral convolution contains area 6 and a fragment in which a few Betz cells may be seen. In the inferior portion of the precentral convolution cortex

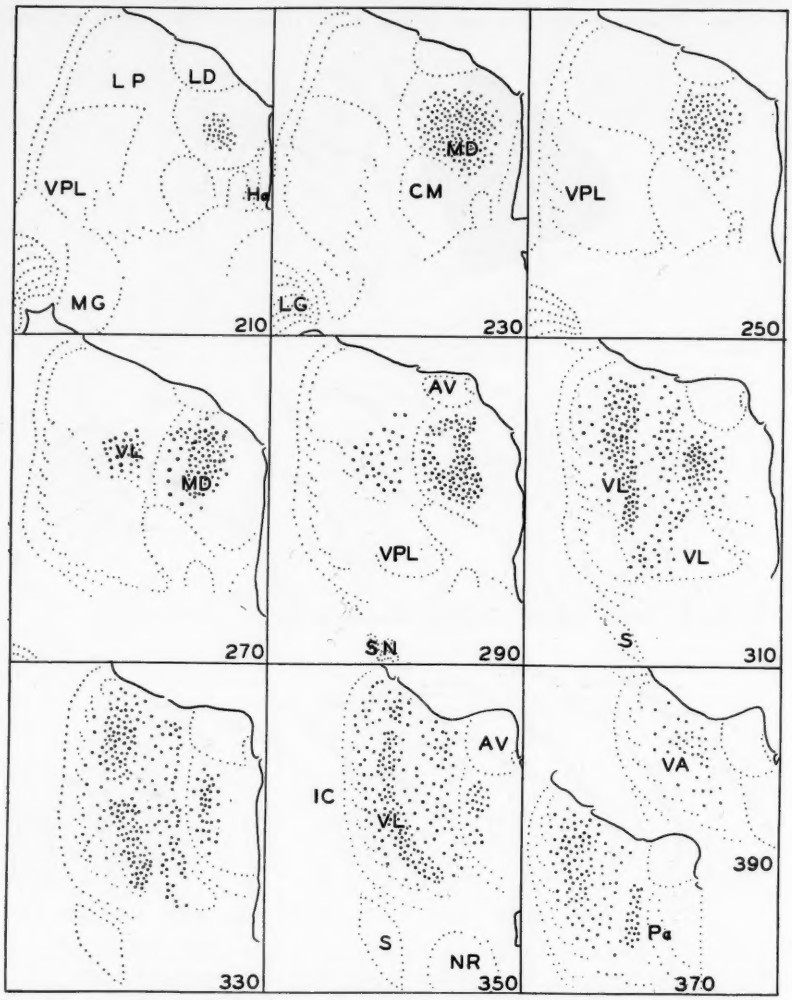

Fig. 5 (Exp. 1, chimpanzee "Mussai"). Representative sections of the left thalamus showing the site and extent of the retrograde cell degeneration.

of both areas 4 and 6 is present, but the former is represented in a relatively few sections. The anterior limit of the lower part of area 4 appears to be the sulcus subcentralis anterior. In the inferior portion of the postcentral convolution is a small lesion damaging 3–4 sq. mm. of cortex. The orbital surface of the frontal lobe

is uninjured; the medial surface of the frontal lobe is undamaged below the callosomarginal sulcus.

Left thalamus. An examination of the serial sections of the left thalamus reveals that the degeneration is confined to the nucleus ventralis lateralis and the nucleus medialis dorsalis. In this case, which survived several years after the initial operation, the thalamic degenerations are not so clearly defined as in the cases which survived no more than 6 weeks to 2 months. The gliosis is less prominent and the site of the degeneration is marked by an absence of neurons in areas where they are normally abundant. There is, however, no question that these two nuclei are markedly degenerated, but both contain areas with normal cells. The changes in the nucleus medialis dorsalis begin in the centre of the nucleus a short distance anterior to its caudal extremity and continue throughout its length. In the middle third the degeneration is mainly confined to the central portion with a fringe of normal cells about the margins. More anteriorly the degeneration is present in the lateral half of the nucleus with relatively little involvement of the medial half. With the advent of the nucleus paracentralis the degeneration diminishes and rapidly disappears. The degeneration of the nucleus ventralis lateralis begins shortly rostral to the middle of the nucleus centrum medianum as an area with a decrease in neurons. Traced anteriorly the area enlarges and at the rostral extremity of the nucleus centrum medianum involves the entire lateral nuclear mass to some extent. Further anteriorly the same patchy cell-less areas are seen almost to the anterior extremity of the lateral nuclear mass, although they become smaller and less pronounced in the anterior part of the thalamus (Fig 5).

This experiment demonstrates that the thalamic projection to the frontal lobe is derived from two thalamic nuclei, the medialis dorsalis and the ventralis lateralis. The degeneration in the nucleus ventralis lateralis differs little in the two thalami, but the changes in the nucleus medialis dorsalis are more severe in the right than in the left thalamus. This may be correlated with the greater extent of the lesion of the right frontal pole, while the lesion of the motor and premotor areas on the two sides is about the same. Further evidence of this relationship is presented by the findings in an animal in which a bilateral ablation of the frontal association areas was carried out (areas 9, 10, 11 and 12 of Brodmann).

Exp. 2. *Successive ablations of both prefrontal areas; severe bilateral degeneration of the nucleus medialis dorsalis; slight changes in the medial portion of the nucleus ventralis lateralis. Weight* 26 *kg.* "*Lucy.*"

The subject of this experiment was a mature female chimpanzee. After more than a year's training in psychological problems by Dr C. F. Jacobsen the left frontal association areas of this chimpanzee were ablated on 16 March 1934. On 25 May 1934 the same areas were removed on the right side. On 24 September 1937 the animal was sacrificed, the body perfused with formalin, and the brain and spinal cord then removed and placed in this solution.

Anatomical examination

Right cerebral hemisphere. The right cerebral hemisphere presents a large lesion of the frontal pole. The cortex of the temporal, occipital and parietal lobes appears normal. Except for two discoloured spots in the frontal operculum, each 0·4 cm. in diameter, the precentral convolution is undamaged. The posterior margin of the ablation extends inferiorly from a point 3·5 cm. anterior to the central sulcus at the midline, crosses the superior frontal sulcus 2·3 cm. anterior to the central sulcus and

the inferior precentral sulcus 1·5 cm. from the central sulcus. On the inferior surface of the hemisphere the line of ablation crosses the orbitofrontal and orbital sulci 0·7 cm. anterior to the tip of the temporal lobe and passes medially to the midline 2 cm. anterior to the optic chiasm. On the medial aspect of the hemisphere, the ablation runs almost vertically downward through the anterior margin of the callosomarginal sulcus and infringes slightly upon the callosal gyrus. The surface of the ablated area is covered by a glial scar and occasional small arachnoidal cysts. The anterior horn of the lateral ventricle is covered by only a thin membrane (Fig. 6).

Examination of serial sections cut coronally through the entire lesion shows that the lesions of the opercular portion of the precentral convolution are quite superficial, probably the result of small arachnoidal adhesions. The posterior margin of the lesion inferiorly is bound by cortex of area 6*bβ*, but a short distance anteriorly it extends into the granular frontal cortex lying along the inferior lip of the inferior frontal sulcus. At about the same level the superior part of the lesion is seen to be bordered by cortex of area 6*ab* lying on the margins of the superior precentral

Fig. 6 (Exp. 2, chimpanzee "Lucy"). The cortical ablations of the prefrontal areas in Exp. 2.

sulcus. In the depth of this sulcus granular frontal cortex is present, suggesting that only a relatively small portion of area 6 has been removed. The lesion follows the superior lip of the callosomarginal sulcus to the rostral extremity of the corpus callosum, at which point it crosses that sulcus and damages the allocortex of the cingular gyrus. The inferior margin of the lesion on the orbital surface of the frontal lobe is bounded by granular cortex. Just rostral to the corpus callosum, the lesion cuts across the olfactory sulcus and the rostral sulci on the medial surface of the lobe (Fig. 7). The ablation has extirpated the greater part of the frontal granular cortex, although small amounts remain on the orbital surface of the frontal lobe and in the depths of the prefrontal sulci.

The right thalamus. Degeneration is present in the nuclei medialis dorsalis and ventralis lateralis. The remainder of the thalamic nuclei are entirely normal. The entire medial nucleus of the thalamus is smaller than normal throughout its extent. Although many normal cells remain, the nuclei shows considerable degeneration. Beginning slightly anterior to its caudal extremity a vertical linear zone of cellular alteration appears in the middle portion of the nucleus medialis dorsalis. Traced anteriorly it increases slightly in size, but does not at any level involve the entire nucleus. Near the oral extremity it parallels the internal medullary lamina to the pole of the nucleus. At this anterior level several discrete areas of

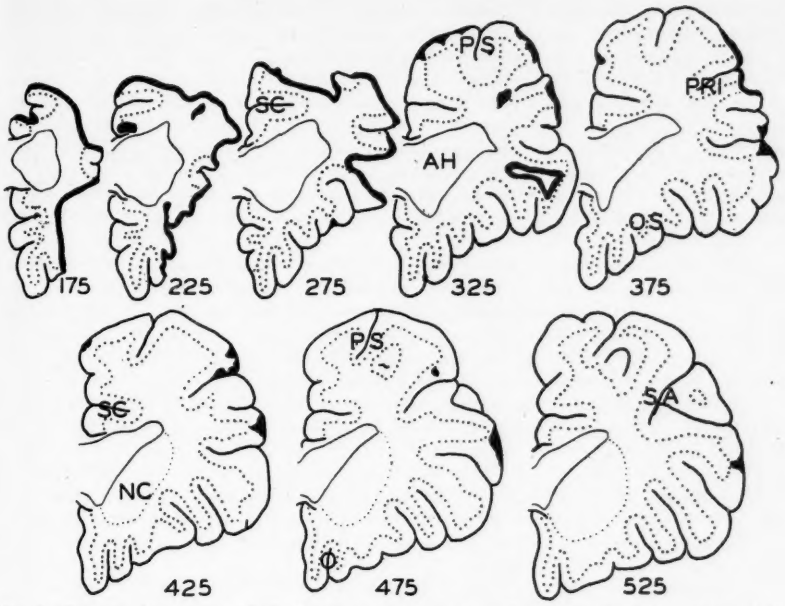

Fig. 7 (Exp. 2, chimpanzee "Lucy"). Serial sections coronally cut of the lesion of the right cerebral hemisphere. In this and the remainder of the illustrations of the cortical sections the most posterior ones are in the lower right corner, and the most anterior in the upper left.

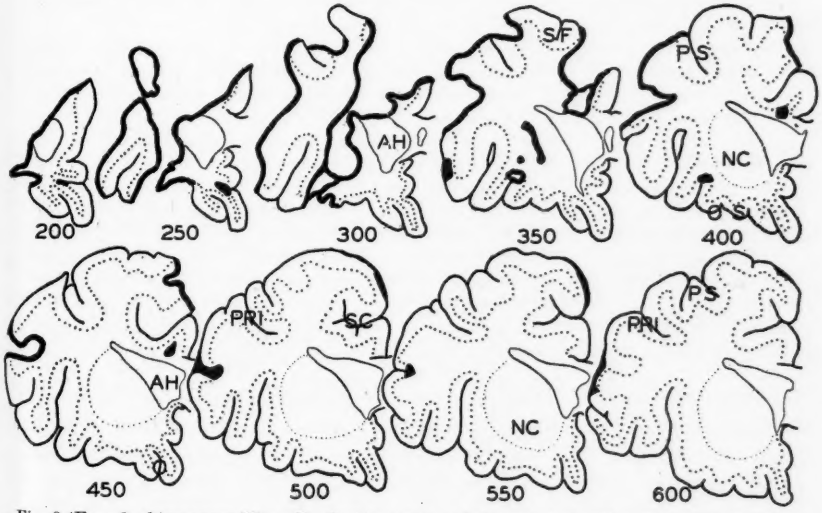

Fig. 8 (Exp. 2, chimpanzee "Lucy"). Serial sections of the left cerebral hemisphere to show the extent of the lesion. The sections were cut in a coronal plane.

degeneration appear in the nucleus ventralis lateralis near the internal medullary lamina. Superiorly a zone is present just lateral to the nucleus anteroventralis, and inferiorly another lies medial to the mammillothalamic tracts. Shortly anteriorly these two zones of gliosis coalesce along the internal medullary lamina adjacent to the anteroventral nucleus. In it no normal neurons are present; it is impossible to be certain how much of this gliosis is due to degeneration of fibres passing in the internal medullary lamina from the nucleus medialis dorsalis, and how much to actual cellular degeneration. Because in the normal thalamus some cells are present in this region, a certain amount of degeneration must be present. The gliosis, gradually diminishing, may be traced to the anterior extremity of the nucleus ventralis lateralis (Fig. 9).

Left cerebral hemisphere. A large lesion of the frontal pole is present, but otherwise the cortex of the hemisphere appears normal. The anterior inferior corner of the gyrus in front of the sulcus subcentralis anterior has been removed in the ablation of the prefrontal areas. The margin of the lesion reaches from a point 3·3 cm. anterior to the central sulcus at the midline, crosses the superior precentral sulcus 2 cm. anterior to the central sulcus, and passes obliquely inferiorly and posteriorly to the anterior margin of the sulcus subcentralis anterior where the margin is 0·5 cm. from the central sulcus. The margin of the ablation crosses to the orbital surface of the frontal lobe 0·2 cm. anterior to the tip of the temporal lobe, and passes to the midline 2·3 cm. from the optic chiasm. The olfactory tract is present but shrunken. On the medial surface of the hemisphere the line of incision crosses the callosomarginal sulcus to damage the anterior margin of the callosal gyrus, and then passes vertically inferiorly to the orbital surface of the lobe. The anterior horn can be seen through a thin glial membrane (Fig. 6).

Examination of serial sections of the lesion show that the inferior angle of the ablation has extended into the cortex of area $6b\beta$, and slightly into area $6a\alpha$. These cortical areas bound the lesion below the superior precentral sulcus. Above this the lesion is bounded posteriorly also by area 6 cortex to the callosomarginal sulcus. At the inferior extremity of the lesion along the orbital surface of the frontal lobe, granular cortex bounds the lesion. In the depth of the superior and inferior frontal sulci on the lateral surface of the frontal lobe small amounts of granular cortex are also present. The ablation has removed most of the frontal granular cortex, leaving only a few fragments on the lateral surface of the hemisphere and some on the orbital surface, but has damaged a considerable area of the premotor cortex inferiorly and a smaller area superiorly (Fig. 8).

The left thalamus. The degeneration in the left thalamus parallels that in the right, but is definitely more extensive. It extends as a vertical zone from near the caudal, to the oral pole of the nucleus medialis dorsalis. Normal neurons are present both along the midline and the internal medullary lamina, but in the intermediate zone practically no normal nerve cells are present. Near the rostral extremity of the main medial nucleus the cells of the nucleus ventralis lateralis lying along the internal medullary lamina are degenerated. This zone of degeneration extends along the entire anterior fourth of the internal medullary lamina to the rostral extremity of the nucleus ventralis lateralis. It is more extensive than on the right side although in the same relative position (Fig. 9).

The severe degeneration in the nucleus medialis dorsalis and its paucity in the lateral nuclear mass show conclusively that the former projects to the prefrontal granular area, while the anterior moiety of the lateral nuclear mass sends its fibres to the motor and premotor areas. The projection to both of these areas, however, is well organized. The following experiments illustrate this fact.

Exp. 3. *Successive ablations of the left motor and premotor leg areas, and right motor leg area; marked degeneration along the lateral margin of the nucleus ventralis lateralis, more pronounced on left than on the right side. Weight* 12 *kg.* "*Yama.*"

The subject of this experiment was an immature male chimpanzee (Exp. 16, Fulton & Keller (1932) and Exp. 8, Fulton & Kennard (1934)). On 21 January 1932

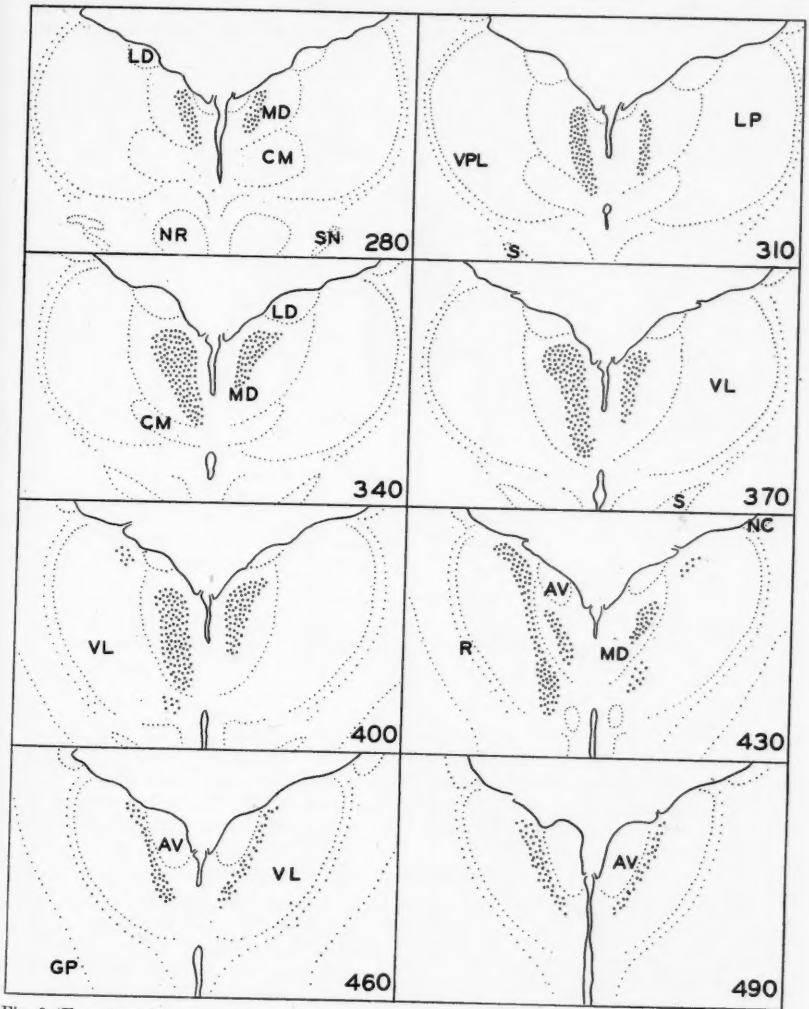

Fig. 9 (Exp. 2, chimpanzee "Lucy"). Representative sections of the thalamus to illustrate the site of the retrograde cell degeneration. In each drawing the right thalamus is to the right and the left to the left.

under sodium amytal anaesthesia a large left osteoplastic flap was reflected. The leg portion of area 4 was removed. The animal had an uneventful convalescence and on 4 March 1932 under sodium amytal a right osteoplastic flap was reflected and the leg portion of area 4 removed. On 16 December 1932 a secondary craniotomy was carried out on the left side and the portion of area 6 anterior to the leg representation of area 4 was removed. On 12 July 1935 the animal was sacrificed.

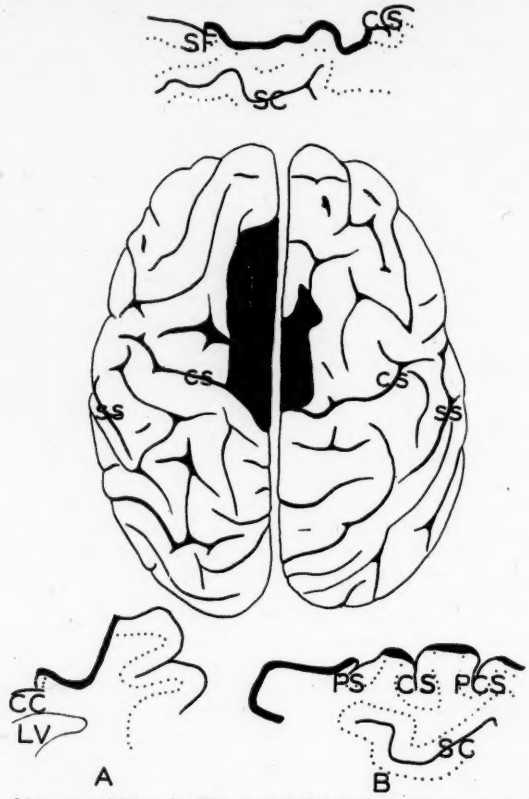

Fig. 10 (Exp. 3, chimpanzee "Yama"). The cortical ablations of the leg areas in Exp. 3. Above a representative sagittal section from the lesion on the right side; below: *A*, a coronal section from the anterior part of the lesion; *B*, a sagittal section from the posterior half of the lesion.

Anatomical examination

Right cerebral hemisphere. The left cerebral hemisphere appears slightly larger than the right, but the convolutions of the latter are full and symmetrical except for a defect in the superior portion of the precentral convolution. This extends 2 cm. anterior to the central sulcus and 1 cm. laterally from the sagittal sulcus. It does not quite reach to the callosomarginal sulcus on the medial surface of the brain, about 0·5 cm. of cortex intervening. The base is 0·8 cm. below the surface of the cortex and covered by a thin arachnoidal membrane. The remainder of the right cerebral hemisphere is quite free from any lesion except for a small abrasion in the superior parietal

region made in the removal of the brain from the calvarium. Microscopic examination of sections confirms the site of the lesion. The anterior margin is in the cortex of area 6 and the posterior adjoins area 3. The cortex of the walls of the callosomarginal gyrus have been entirely spared, but no cortex of area 4 type is visible. The lesion is then practically confined to the cortex of area 4 (Fig. 10).

Thalamus. The right thalamus has been cut sagittally in serial sections. Every fifth section has been stained with thionin and mounted. An examination of these sections shows beautifully the division of the thalamic nuclei. The distinct cell structure of the nucleus ventralis posterior is well demonstrated, and the cellular morphology and architecture of the nucleus ventralis lateralis is also well defined,

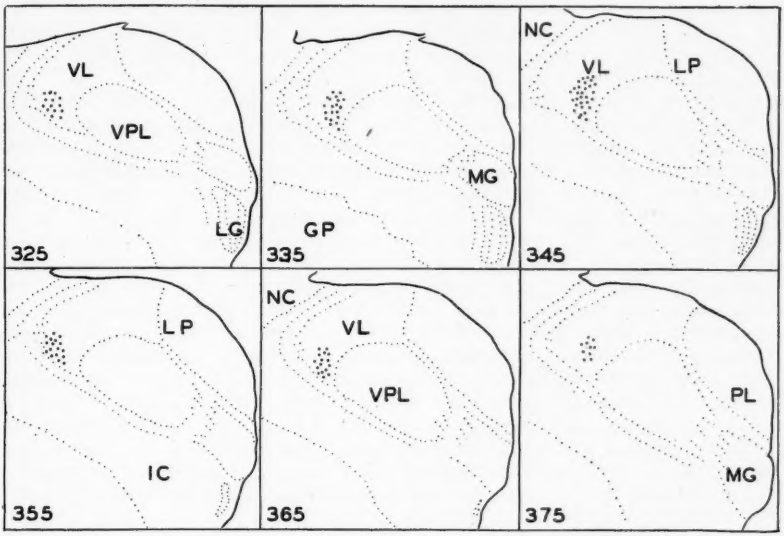

Fig. 11 (Exp. 3, chimpanzee "Yama"). Representative sagittal sections of the right thalamus to show the site of the retrograde cell degeneration. It is easy to determine the boundaries of the lateral nuclear mass in these sections. The most lateral sections are in the upper left-hand corner.

and the borders between these two nuclei are distinct. The border between the nuclei ventralis lateralis and lateralis posterior is likewise conspicuous. It is evident from these sections that the nucleus ventralis lateralis extends posteriorly and dorsally over the nucleus ventralis posterior. The degeneration is present in quite a limited area, less than 1·5 cm. in width (about 70 sections cut at 20 m.). It is situated in the nucleus ventralis lateralis just anterior to the nucleus ventralis posterior along the external medullary lamina. At its posterior margin it almost reaches the dorsal tip of the nucleus ventralis lateralis. Further anteriorly it becomes more ventrally situated, but maintains its peripheral position along the external medullary lamina, just anterior to the margin of the nucleus ventralis posterior. In the involved area the degeneration is almost complete, with an occasional cell remaining. A careful examination of the remainder of the thalamus including the geniculate bodies, anterior and medial nuclei shows no evidence of retrograde cell degeneration (Fig. 11).

Left cerebral hemisphere. The occipital and temporal lobes and the frontal pole appear normal. The convolutions of the pre- and postcentral superior, middle and

inferior frontal convolutions, and the parietal lobules are severely atrophied. These convolutions are approximately 25% smaller than those on the right side. The arachnoid over these areas is thickened, milky and stringy. There is a large defect in the leg portion of the motor and premotor areas. It extends 4·5 cm. anterior to the central sulcus, 1·5 cm. from the sagittal sulcus, and on the medial surface of the hemisphere reaches anteriorly to within 3–4 mm. of the corpus callosum. Near the central sulcus it is shallower and has not damaged the cingular gyrus. The base of the lesion is covered by a thin arachnoidal membrane. For 2 cm. near the midline the anterior margin of the postcentral convolution has been injured superficially.

Study of sections microscopically shows that the anterior portion of the lesion has extended medially to within a few millimetres of the corpus callosum, and left only a small portion of the cingular gyrus. It has not, however, encroached upon the lateral ventricle. Posteriorly the lesion is more superficial and the margins of the callosomarginal sulcus have been spared. The posterior margin of the lesion abuts the cortex of area 3 on the anterior wall of the central sulcus. A small lesion damaging all layers of the cortex is present on the convexity of the postcentral convolution. The cortex adjoining the lesion shows chronic cell change and arachnoidal hyperplasia, but is not involved by softening or other vascular lesion. The lesion has removed the superior portions of areas 4 and 6, and damaged slightly the prefrontal granular cortex anteriorly and the cortex of the postcentral convolution posteriorly. The remainder of the cortex is little changed (Fig. 10).

Left thalamus. A careful examination of the serial sections of the left thalamus shows one large area of cellular degeneration in the lateral portion of the nucleus ventralis lateralis, and a second small area of diffusely scattered degeneration in the lateral part of the nucleus medialis dorsalis. The sections of the thalamus have been cut sagittally so that it is easy to distinguish the divisions of the lateral nuclear mass. Beginning along the external medullary lamina just rostral to the anterior extremity of the nucleus ventralis posterior is a small scattered area of degeneration which, although always maintaining its lateral position, when traced anteriorly is seen to enlarge and become more intense. Posteriorly it is situated dorsal to the nucleus ventralis posterior, but further anteriorly where that nucleus is no longer present the degeneration extends both dorsally and ventrally to form a narrow zone of cellular degeneration along the external medullary lamina. In the rostral portion of the nucleus ventralis lateralis the degeneration decreases, but a zone of gliosis continues anteriorly along the external medullary lamina for a considerable distance (100 sections—2 mm.). Whether this zone represents true degeneration of neurons or only gliosis due to degeneration of the fibres cannot be stated (Fig. 12).

Examination of the main medial nucleus reveals a small zone of retrograde cell degeneration just medial to the internal medullary lamina about the middle anteroposteriorly of the nucleus. The area is quite small and does not extend through more than $\frac{1}{2}$ mm.

These results indicate that the thalamic projection to the leg area of the cerebral cortex is derived from the lateral portion of the nucleus ventralis lateralis. It, moreover, suggests that the projection to area 4 is somewhat more intensive than that to area 6, but that the thalamus projects to both areas. The slight degeneration in the nucleus medialis dorsalis of the left thalamus is probably the result of slight injury to the frontal granular cortex.

Further data concerning the topical thalamic projection may be drawn from the following experiment.

Exp. 4. *Ablation of the left arm and leg motor and premotor areas; marked changes in the cells of the lateral half of the left nucleus ventralis lateralis. Weight* 20·5 *kg.* "*Tommy.*"

The subject of this experiment was a male chimpanzee who had been trained by Dr T. C. Ruch to discriminate grades of sandpaper. On 4 November 1935 under sodium amytal anaesthesia a large right osteoplastic craniotomy was performed and the entire postcentral convolution to the depth of the central and postcentral sulci was ablated. The animal had an uneventful convalescence. On 6 December 1935

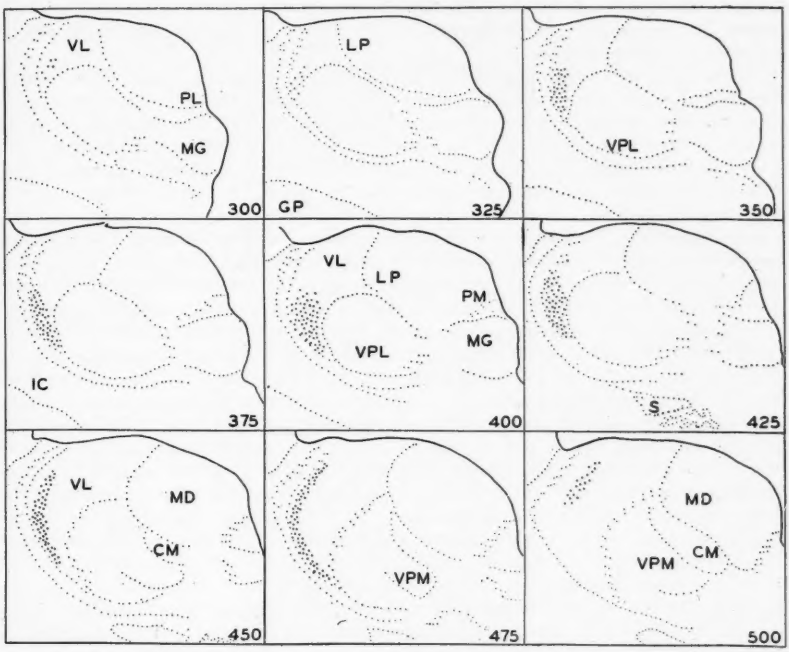

Fig. 12 (Exp. 3, chimpanzee "Yama"). Representative serial sections of the left thalamus to show the extent and position of the retrograde cell degeneration. The sections are cut in a sagittal plane.

under sodium amytal anaesthesia a left-sided flap was reflected and the upper two-thirds of the motor and premotor areas ablated. The convalescence was uneventful. The animal was still able to discriminate grades of sandpaper with the left upper extremity, and therefore on 7 February 1936 under sodium amytal anaesthesia the right osteoplastic flap was re-elevated, and the remainder of the right parietal lobe to parieto-occipital sulcus was removed. The wound became infected and drained for several weeks, but the animal showed little systemic reaction, and no evidence of meningitis. On 14 May 1936 the animal was sacrificed under ether and the vascular system was perfused with saline and then alcohol.

Anatomical examination

Left cerebral hemisphere. The dura mater is loosely adherent to the margins of the ablated area. The only lesion is in the superior precentral region extending anteriorly from the central sulcus inferiorly 2 cm. and at the midline 3 cm. The posterior border of the lesion follows the central sulcus to the angle at which the latter turns inferiorly to approach the sylvian fissure. The anterior margin passes obliquely superiorly and anteriorly across the inferior precentral sulcus and the posterior portion of the superior frontal sulcus. The crater of the lesion is 0·6 cm. below the surface of the cortex, and its margins slope anteriorly and posteriorly. The postcentral convolution is widened and the cortex on the posterior lip of the central sulcus is exposed but appears to be uninjured. The lesion extends on the medial surface of the hemisphere to the callosomarginal sulcus but does not involve the cortex of the cingular gyrus. At the midline a small fringe of cortex remains on the anterior lip of the central sulcus. The remainder of the cortex on the lateral surface of the left cerebral hemisphere is normal (Fig. 13).

Serial sections of this hemisphere cut almost in a sagittal plane show clearly the extent of the lesion. The posterior margin extends along the central sulcus. In its superior part the ablation is bounded by cortex of type 3*a*, and it seems probable that at least a part of this type of cortex has been removed in the ablation. The cortex on the inferior lip of the callosomarginal sulcus is entirely spared by the ablation and there is no damage to the cingular gyrus. The cortex of the superior lip is entirely lacking. Along the central sulcus an occasional gigantopyramidal cell is seen, but in no place is there a significant amount of area 4 cortex. The posterior margin of the lesion is largely bounded by area 3 cortex at the trough of the central sulcus. The posterior wall of the sulcus is not damaged by the ablation. Area 3 bounds the lesion posteriorly to the knee of the central sulcus, at which point the ablation passes through the frontal convolutions and its margin is formed by cortex of area 6, except near the midline where granular frontal cortex bounds it (Fig. 14).

Left thalamus. A careful examination of serial sections of the thalamus shows that the degeneration is confined to the nuclei ventralis lateralis and medialis dorsalis. The slight degeneration in the latter nucleus consists of a zone lying along the internal medullary lamina just inferior to the nucleus lateralis dorsalis in the middle third of the nucleus medialis dorsalis. It does not extend into the anterior third of the thalamus. Although an occasional normal cell remains in the involved area the degeneration is clear and unmistakable. In the nucleus ventralis lateralis the degeneration is both more extensive and intensive. It begins posteriorly as a laterally situated zone of degeneration above the nucleus ventralis posterior. Traced anteriorly it becomes larger to include all the lateral portion of the nucleus ventralis lateralis. At the oral extremity of the nucleus ventralis posterior the degeneration reaches the inferior margin of the lateral nuclear mass along the external medullary lamina. It thus involves the external half of the nucleus ventralis lateralis and completely spares the most medial portion, approximately the medial half. Traced anteriorly the degeneration maintains the same relative position along the external portion of the nucleus. The most dorsal and medial portion of the nucleus is little involved. At the rostral extremity of the lateral nuclear mass the degeneration disappears and the normal cells of the nucleus ventralis anterior remain. The nuclei anteroventralis and anterodorsalis, which are quite distinct in this specimen, are normal (Fig. 15).

This experiment further substantiates the contention that a definite spatial organization exists in the projection to the cerebral cortex from the nucleus ventralis lateralis. The radiation is so arranged that the lateral portion of the nucleus projects to the leg motor area, the medial part to the face region, and the intermediate zone of the ventral lateral nucleus to the arm motor area.

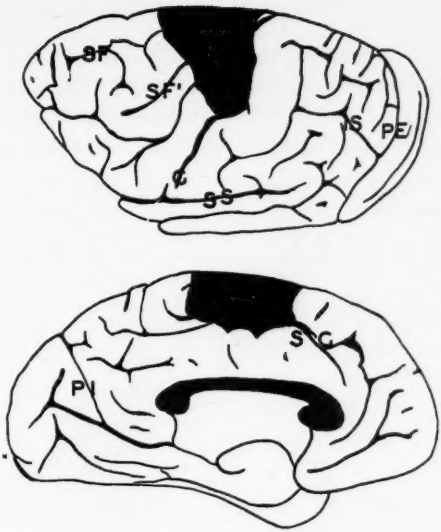

Fig. 13 (Exp. 4, chimpanzee "Tommy"). The left hemisphere of chimpanzee "Tommy" to show the extent of the cortical ablation above on the lateral surface of the hemisphere, and below, on the medial surface.

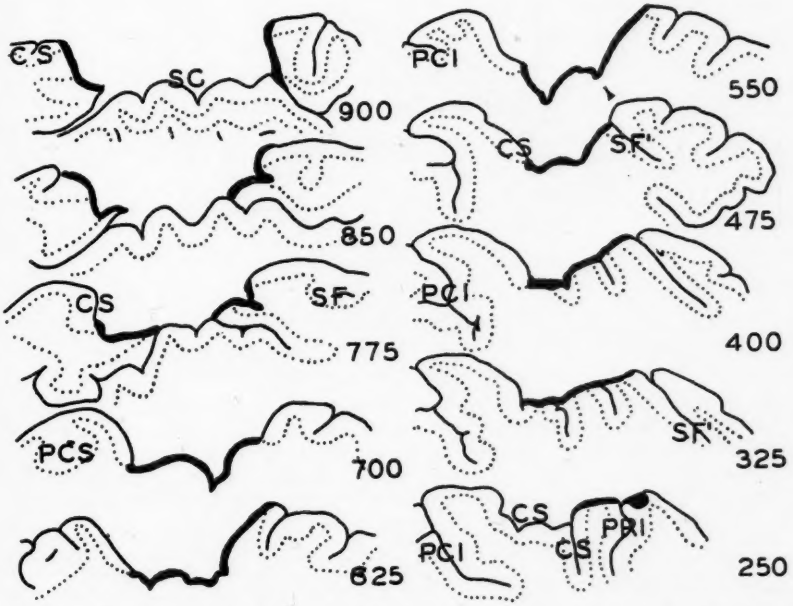

Fig. 14 (Exp. 4, chimpanzee "Tommy"). Serial sections of the frontal lobe to show the extent of the ablation of the left motor and premotor areas. The sections are cut in a sagittal plane

(2) The parietal lobe

Despite the fact that the parietal lobe has been considered the cortical sensory representation, until recently relatively little has been established concerning the organization of its afferent fibres. In the macaque monkey the

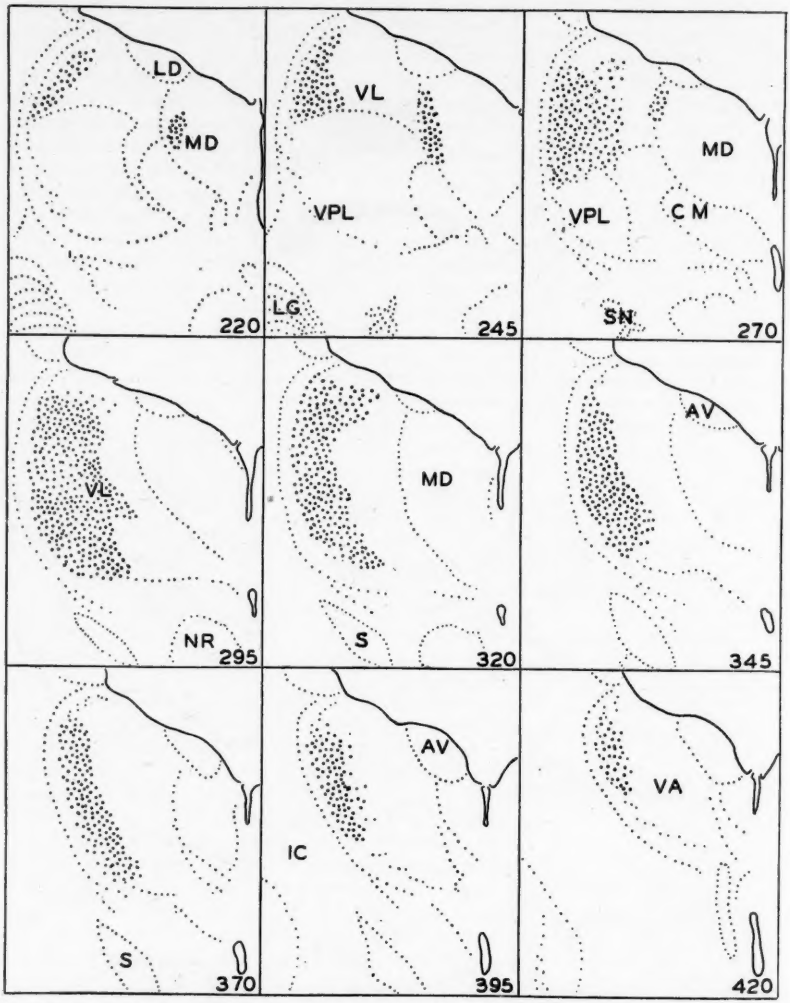

Fig. 15 (Exp. 4, chimpanzee "Tommy"). Coronal serial sections of the left thalamus of chimpanzee "Tommy" to show the site and position of the retrograde cell degeneration.

thalamic projection to this part of the cerebral cortex has been shown to have a precise spatial organization. The following experiments indicate that a similar arrangement is present in the chimpanzee.

Exp. 4 (*cont.*). *Successive ablations of the right parietal lobe with slight encroachment on the motor area; severe degeneration in the pulvinar, nuclei ventralis posterior, lateralis posterior and slight changes in the nuclei lateralis dorsalis and ventralis lateralis.* "*Tommy.*"

Anatomical examination

Right hemisphere. The dura over the right cerebral cortex was adherent over the superior portion of the ablated area, but was reflected after fixation of the brain without difficulty. There is one large lesion of the parietal lobe extending from the central sulcus to the external parieto-occipital fissure. Along the inferior portion of the central sulcus only the postcentral convolution is removed, the supramarginal gyrus being spared. The lesion measures 2·1 cm. anteroposteriorly, and extends laterally 3·1 cm. from the midline. Along the inferior portion of the central sulcus

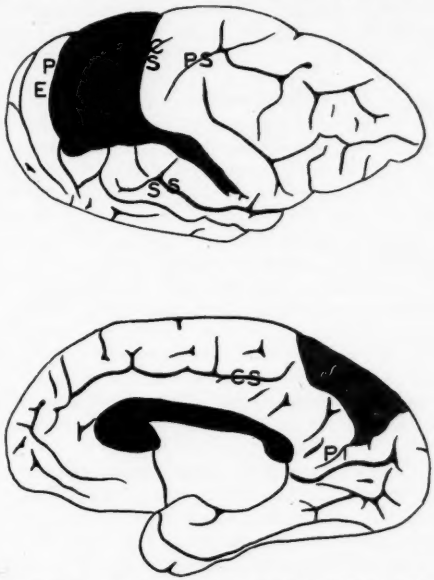

Fig. 16 (Exp. 4, chimpanzee "Tommy"). The site and position of the parietal lobe extirpation of the right cerebral hemisphere of chimpanzee "Tommy". Above the lateral surface and below the medial surface of the hemisphere; in the latter CS—sulcus callosomarginalis.

it is 0·4 cm. in width. The inferior part of the angular and the supramarginal gyri are intact. The cortex of the posterior wall of the external parieto-occipital sulcus also appears to be undamaged. The precentral convolution is markedly widened but in its superior portion there is no evidence of softening or injury. At the level of the inferior precentral sulcus the cortex of the precentral convolution is discoloured and softened for a distance of 0·6 cm. anterior to the central sulcus. This lesion of the precentral convolution reaches almost to the inferior extremity of the central sulcus. The remaining cortex of the right cerebral hemisphere appears normal. On the medial surface of the hemisphere the lesion extends from the internal parieto-occipital sulcus anteriorly to the ascending ramus of the callosomarginal sulcus. It reaches 0·8 cm. on the medial surface to the superior lip of the callosomarginal sulcus. It has not damaged the pericalcarine cortex, nor the cortex of the posterior part of the cingular gyrus (Fig. 16).

A careful study of serial sections of the right parietal lobe shows that the anterior margin of the lesion extends along the central sulcus with varying amounts of damage to the precentral convolution. On the medial surface of the hemisphere the lesion has extended almost to the posterior prolongation of the callosomarginal sulcus, but has not damaged the cingular gyrus. As the sections are cut parallel to the cortex of this convolution it is impossible to state definitely that no damage has been done to the allocortex in this region. Along the midline where the central sulcus is superficial the lesion is bounded anteriorly for a centimetre by cortex of area 3. Below this, area 4 cortex forms the anterior boundary of the lesion. A few millimetres inferiorly the cortex of area 4 appears sclerotic and its posterior part is devoid of large Betz cells although such cells are seen on the surface of the convolution. This sclerosed area 4 bounds the lesion to the angle of the central convolution. At this point, where it is normally narrow, no area 4 cortex remains, although area 6 immediately anterior is unchanged. Normal area 4 cortex is absent for only 2–3 mm., below which Betz cells are seen again. The damage to area 4 in the lower portion of the precentral convolution is relatively slight although the lesion borders on this type of cortex to its inferior extremity, which does not reach the sylvian fissure (Fig. 17).

The posterior margin of the ablation extends along the external parieto-occipital fissure, but in no point damages the striate cortex which lies within a millimetre or two of the posterior lip of this sulcus. Near the midline the lesion posteriorly is quite superficial, injuring only the cortex on the surface of the hemisphere, and leaving intact that on the walls of the internal parieto-occipital fissure, which extends laterally beneath the occipital operculum for more than a centimetre. A few millimetres below the inferior margin of the latter sulcus, the superior tip of the superior temporal fissure appears. The posterior margin of the lesion to this point borders area 19. The lesion becomes more superficial and considerable cortex along the walls of the superior temporal sulcus remains undamaged. The border of the lesion here is the cortex of the supramarginal gyrus. Just inferior to the tip of the sylvian fissure the lesion in the posterior parietal lobe disappears but anteriorly the ablation may be followed along the postcentral convolution. Below the angle of the central sulcus the lesion is bordered by distorted cortex of type 2. This cortical type borders the posterior margin of the lesion to its inferior tip below which normal cortex of area 3 may be seen.

Right thalamus. Serial sections of the right thalamus show that the degeneration is confined to the pulvinar and lateral nuclear mass. The medial and lateral geniculate bodies, the nuclei medialis dorsalis and anteroventralis are quite normal throughout their extent. Beginning near the caudal pole of the pulvinar as an area of rather diffuse degeneration in the dorsolateral corner, the degeneration rapidly increases in size until at the level of the caudal margin of the medial geniculate body it occupies the greater part of the nucleus pulvinaris lateralis. The inferior nucleus of the pulvinar is practically unchanged, and the nucleus pulvinaris medialis is only slightly involved at this level. Further anteriorly, however, the degeneration extends well into the lateral portion of the nucleus pulvinaris medialis but even here it is not completely degenerated. The most medial portion composed of rather darkly staining cells surrounding the posterior margin of the nucleus medialis dorsalis, remains unchanged. The entire anterior portion of the lateral nucleus of the pulvinar is degenerated. At the caudal pole of the nucleus centrum medianum the degeneration invades the nucleus ventralis posterior. Although a zone of normal cells lies along the nucleus centrum medianum, the normal cytoarchitecture of the nucleus ventralis posterior is entirely absent. In the nucleus lateralis posterior are a few normal looking cells which increase in its more anterior portions. It is difficult to determine the boundary between this nucleus and the rostral portion of the nucleus ventralis lateralis. However, the degeneration in the lateral nuclear mass anterior to the middle of the nucleus

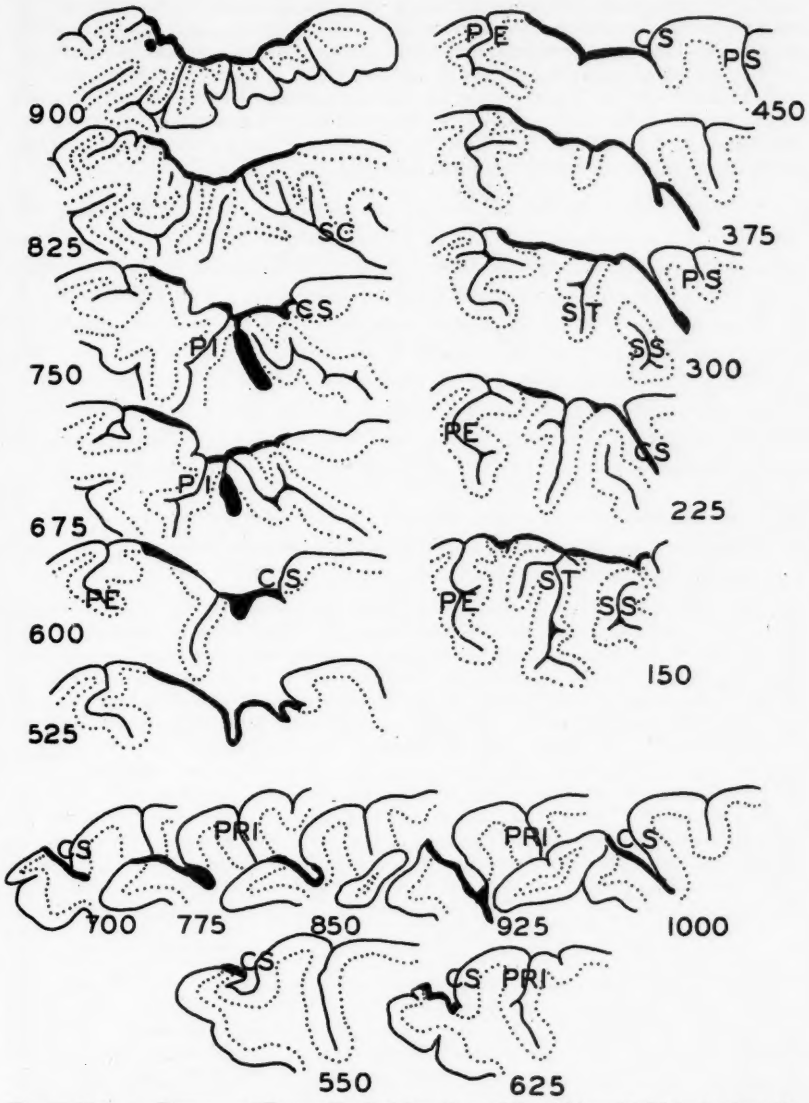

Fig. 17 (Exp. 4, chimpanzee "Tommy"). Serial sections of the right parietal lobe to show the precise limits of the ablation. The upper sections are cut sagittally through the superior portion of the parietal lobe, and the lower ones, at right angles to the central sulcus, from the inferior half of the ablation.

centrum medianum is present in the nucleus ventralis posterior. The latter nucleus is practically completely degenerated except for a small zone bordering the nucleus centrum medianum. In its rostral extremity a few of its characteristic large cells may be seen. The degeneration is not confined to the nucleus ventralis posterior but a scattered degeneration represented by a severe gliosis appears to extend superiorly and anteriorly into the lateral part of the nucleus ventralis lateralis. It can be traced to the middle of the nucleus where the last vestige of degeneration lies along the external medullary lamina. There is slight degeneration in the ventral portion of the anterior half of the nucleus lateralis dorsalis (Fig. 18).

Although the lesion in this case is somewhat more extensive than was intended, it demonstrates that the projection to the frontal and parietal cortex arises from distinct parts of the thalamus. The fibres passing to the motor and premotor areas are the axons of cells lying in the anterior moiety of the thalamus. It is impossible to be certain of the exact extent of the true retrograde cell degeneration in the nucleus ventralis lateralis, for at least a portion of the gliosis represents degeneration of the fibre tracts traversing the nucleus. Since the greater part of the pulvinar degenerated in this experiment, it seems likely that the temporal and occipital lobes receive few fibres from the thalamus proper.

In another animal the ablation was not quite so extensive.

Exp. 5. *Ablation left prefrontal lobe and parietal lobe; patchy degeneration of the medial nucleus and the nuclei ventralis posterior, lateralis posterior and pulvinaris lateralis. Weight* 8·5 *kg.* "*Carrie Nation.*"

This animal was a young female chimpanzee, so unreliable and aggressive that despite repeated attempts, it could not be tamed sufficiently even for an examination. On 25 September 1935 under sodium amytal anaesthesia a large left osteoplastic flap was reflected. After mapping the cerebral cortex, Dr Fulton removed the tip of the frontal lobe 3·5 cm. anterior to the central sulcus. The latter sulcus was then opened and the parietal lobe ablated with the Bovie unit. The cortex from the sylvian fissure inferiorly to the callosomarginal sulcus on the medial surface of the hemisphere was removed. Posteriorly the ablation extended almost to the external parieto-occipital fissure. Five weeks after operation the animal developed a severe impetigo which did not clear up for over a month. On 10 December 1935 under sodium amytal anaesthesia a right osteoplastic craniotomy was performed and lesions made in the postcentral convolution on that side. Unfortunately the animal died 8 hours after operation from aspirating mucus and vomitus. The brain was injected with saline followed by alcohol and the entire central nervous system placed in 95 % alcohol.

Anatomical examination

Right cerebral hemisphere. The fresh operative wounds only were present. No further study was made of this hemisphere.

Left cerebral hemisphere. There is a small extradural encysted abscess under the middle of the bone flap. Aside from this there are no unusual findings in the dura. Two lesions are present in the left cerebral hemisphere, one in the frontal pole and the second in the parietal lobe. The first extends posteriorly 2·5 cm. from the tip of the frontal cortex. It reaches posteriorly almost to the anterior angle of the inferior precentral sulcus, but superiorly does not extend so far caudally. On the medial surface of the hemisphere the lesion reaches to the anterior margin of the callosomarginal sulcus, but the cortex lying on this margin seems to have been spared.

The lesion of the parietal lobe extends posteriorly from the central sulcus, the

cortex on the anterior margin of which appears to be undamaged. The only cortex of the postcentral convolution remaining is a small strip in the superior angulation of the central sulcus, and the inferior part along the sylvian fissure. A strip 0·6 cm. in width remains along the latter sulcus almost to its caudal tip. The lesion extends

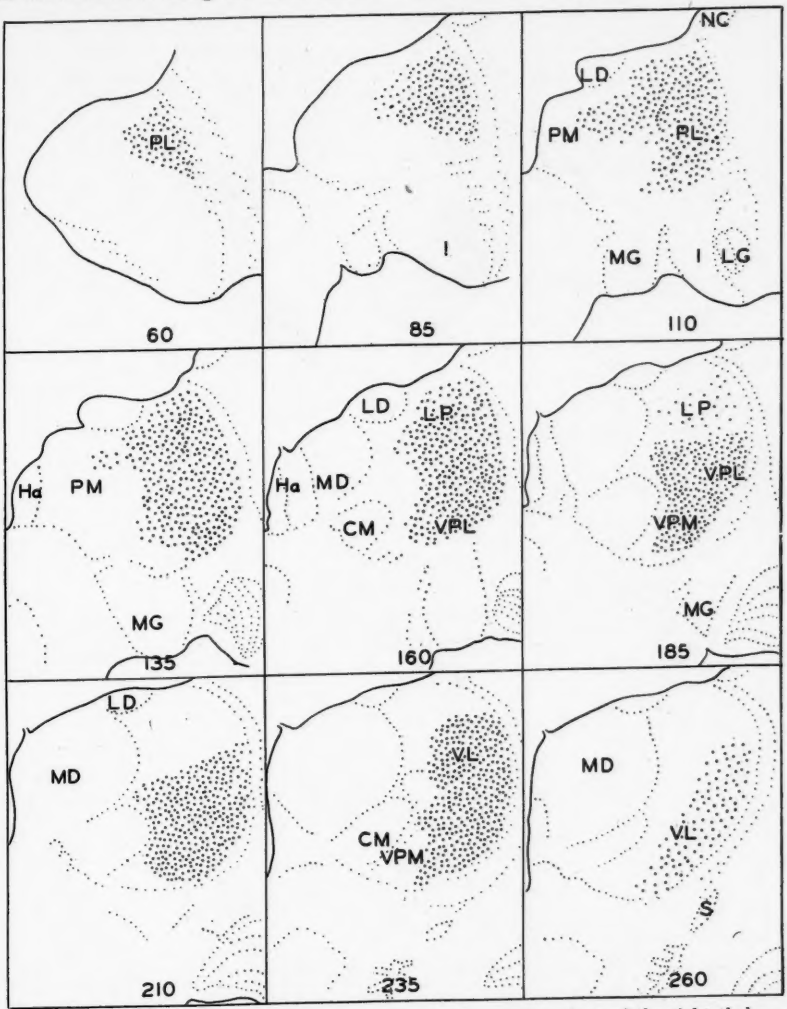

Fig. 18 (Exp. 4, chimpanzee "Tommy"). Representative serial sections of the right thalamus of chimpanzee "Tommy" showing the extent and position of the retrograde cell degeneration.

to within 1 cm. of the parieto-occipital sulcus. Grossly a small part of cortex lying in the depth of the postcentral sulcus appears intact. The lesion extends 0·4 cm. on the medial surface to the superior lip of the callosomarginal sulcus and reaches within 0·5 cm. of the internal parieto-occipital sulcus (Fig. 19).

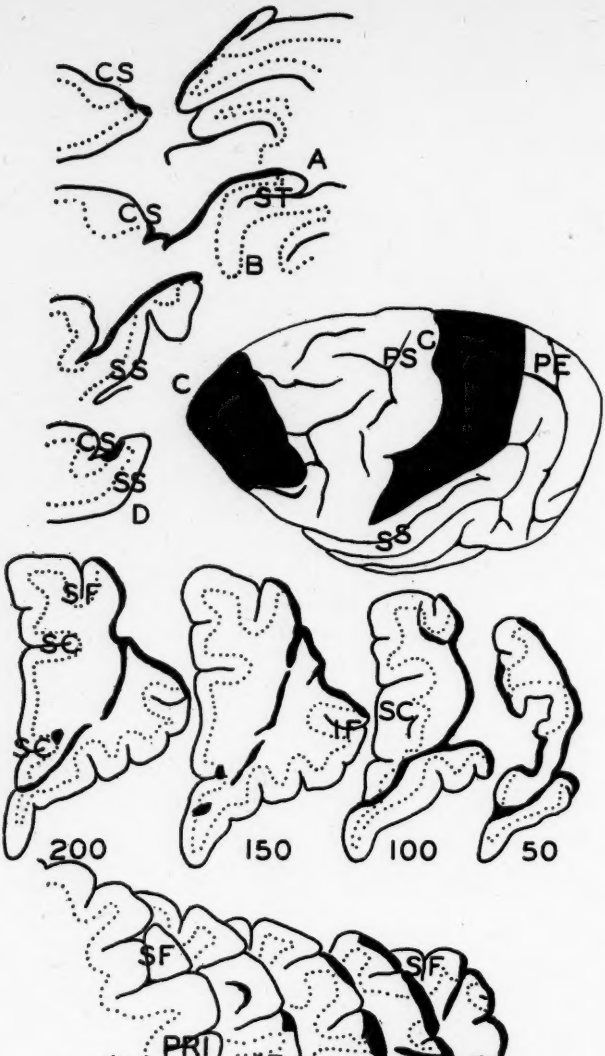

Fig. 19 (Exp. 5, chimpanzee "Carrie Nation"). The left cerebral hemisphere to show the cortical ablations. Below and to the right serial sections of the frontal lobe to illustrate the precise limits of the ablation, and above four representative sections from the parietal lobe lesion. *A*, sagittal section near the hemispheral fissure; *B*, sagittal section just below the intraparietal sulcus; *C*, oblique section at the tip of the sylvian fissure; and *D*, oblique section at the tip of the central sulcus.

The entire cortex containing the lesion of the frontal lobe was taken for serial sections, cut at 25 microns and every 25th section mounted after staining with toluidine blue. The ablated block from the frontal lobe was also sectioned in the same manner. Three blocks were taken from the parietal lobe lesion and representative sections made from these; one block from the lower end of the ablation was sectioned serially, every 25th section being stained and mounted.

Parietal lobe. An examination of the sections of the parietal lobe shows that the entire postcentral convolution and most of the anterior part of the parietal lobules have been removed. The anterior margin of the ablation is formed by cortex of area 3 along the trough of the central sulcus; in no point does area 4 seem to have been encroached upon. The lesion has reached to the callosomarginal sulcus on the medial surface of the hemisphere, and removed the cortex of the superior lip of this sulcus. The posterior margin of the ablation is formed by cortex of areas 5 and 7, except at the inferior part where cortex of type 2 forms a part of the border (Fig. 19).

Frontal lobe. An examination of the serial sections of the ablated tissue shows that it is composed of frontal granular cortex except for a small corner in which tissue of types $6b\beta$ and $6a\alpha$ can be recognized. This part of the block was removed from the posterior part of the frontal lobe, i.e. from the anterior part of the face region. An examination of serial sections of the frontal lobe shows that the entire tip of the latter has been removed, the margins of the ablation being frontal granular cortex. Slightly posteriorly the lesion recedes from the medial aspect of the frontal lobe, and is entirely confined to its convexity. At this point the superior margin of the ablation is bounded by granular frontal cortex. The latter type of cortex forms the margin of the ablation along the superior lip of the inferior frontal sulcus. The lesion terminates at the junction of the inferior frontal sulcus with the inferior precentral sulcus. A few islands of cortex (area 6) along the anterior branches of the precentral sulcus are present in the posterior part of the lesion, but except for these the cortex of the lateral surface of the frontal lobe above the inferior frontal sulcus and anterior to the inferior precentral sulcus has been largely removed.

Left thalamus. An examination of the serial sections of the left thalamus reveals severe retrograde cell degeneration in the pulvinar and lateral nuclear mass. Posteriorly it begins in the dorsolateral corner of the pulvinar. Slightly caudal to the junction of the pulvinar and the mesencephalon, the degeneration is scattered and diffuse with many normal cells remaining. Slightly anteriorly the scattered areas become confluent to form a large zone of degeneration in the dorsal half of the pulvinar. Along the stratum zonale is a layer of normal cells, and through a number of sections a thin zone of normal cells stretches mediolaterally across the middle of the pulvinar through the degenerated area. The nuclei medialis dorsalis and lateralis dorsalis making their appearance at this level show no cellular alterations. At the caudal level of the nucleus centrum medianum, the degeneration involves all the nuclei ventralis posterior and lateralis posterior above the corticotectal tracts except the dorsal layer of cells along the stratum zonale. It clearly outlines the nucleus centrum medianum. In this degenerated zone only an occasional normal appearing cell persists. The medial geniculate body and inferior nucleus of the pulvinar show no changes. At this level a fairly broad linear zone of retrograde cell change appears in the nucleus medialis dorsalis extending from just dorsal to the habenulo-interpeduncular tract to the level of the taenia thalami. While the margins of this area merge with the normal portion of the nucleus, the degenerated part contains few normal cells.

The normal cytoarchitectural characteristics of the caudal part of the nucleus ventralis posterior are entirely obliterated by the severe degeneration. Scattered about are a few normal cells. Only a few degenerated cells are present in the anterior part of the nucleus lateralis posterior. It is difficult to determine in this preparation the exact boundary between the dorsal portion of the nucleus ventralis lateralis and

the nucleus lateralis posterior. The degeneration extends anteriorly along the ventral and lateral margin of the lateral nuclear mass to the rostral extremity of the nucleus centrum medianum. In no place can the cellular characteristics of the nucleus ventralis posterolateralis be recognized; thus it seems likely that the degeneration extends slightly into the nucleus ventralis lateralis, but the greater portion of this nucleus is normal. The linear degeneration in the nucleus medialis dorsalis becomes laterally situated at the anterior extremity of the nucleus. There is considerable gliosis in the anterior part of the internal medullary lamina, probably representing degenerating fibres passing from the main medial nucleus to the frontal lobe (Fig. 20).

A casual comparison of the degenerations in the pulvinar in this experiment and those in the right thalamus of Exp. 4 show that the changes are more extensive in the latter, except that the nucleus pulvinaris medialis is more extensively degenerated in the former. These differences may be correlated with the varied extent of the two lesions. The right parietal lobectomy of the chimpanzee "Tommy" involved more of the posterior parietal and less of the supramarginal cortex than the lesion in Exp. 5. The assumption is then, that the posterior and lateral portions of the pulvinar project to the posterior parietal regions, and the medial part to the supramarginal gyrus. Further evidence will be given for this conclusion later.

The parietal lobe is composed cytoarchitecturally of at least two distinct types of cortex; one, occupying the postcentral convolution and the posterior lip of the central sulcus, shows a predominance of the granular layers, the other found over the parietal lobules is neither granular nor agranular but has all six layers well developed. It would not be surprising then if these distinct cortical types received their thalamic fibres from different nuclei, as the following experiment indicates.

Exp. 6. *Ablation of postcentral convolution; extensive degeneration limited to the nucleus ventralis posterior. Weight* 21·2 *kg.* "*Maggie.*"

The subject of this experiment, a capricious temperamental female chimpanzee, was first operated upon on 7 December 1935 under sodium amytal anaesthesia. A left central osteoplastic flap was reflected, and after mapping the exposed cortex, the principal veins along the central and postcentral convolution were coagulated. A subpial dissection of the entire postcentral convolution was carefully carried out from the callosomarginal sulcus on the medial surface of the hemisphere to the inferior tip of the central sulcus. The ablation extended well into the depth of the central sulcus. The posterior margin of the ablation followed the posterior margin of the postcentral sulcus. The flap was closed in the usual manner. The animal had an uneventful convalescence. On 12 February 1937 the osteoplastic flap was reopened. The precentral convolution and the cortex of the parietal lobules appeared normal and the margins of the lesion were separated only 4–5 mm. The entire parietal lobe to the external parieto-occipital sulcus was ablated en bloc. Unfortunately the animal died at the close of the operation. The brain was immediately injected with formalin.

Anatomical examination

Left cerebral hemisphere. The only lesion in the brain is situated in the left parietal lobe. The cortex of the frontal, occipital and temporal lobes appears undamaged. The anterior margin of the ablation lies along the central sulcus, the posterior wall

of which is removed. The anterior wall does not seem to be at all injured. The lesion extends from the callosomarginal sulcus on the medial surface of the hemisphere to within 2 cm. of the sylvian fissure inferiorly. Its posterior margin is the external

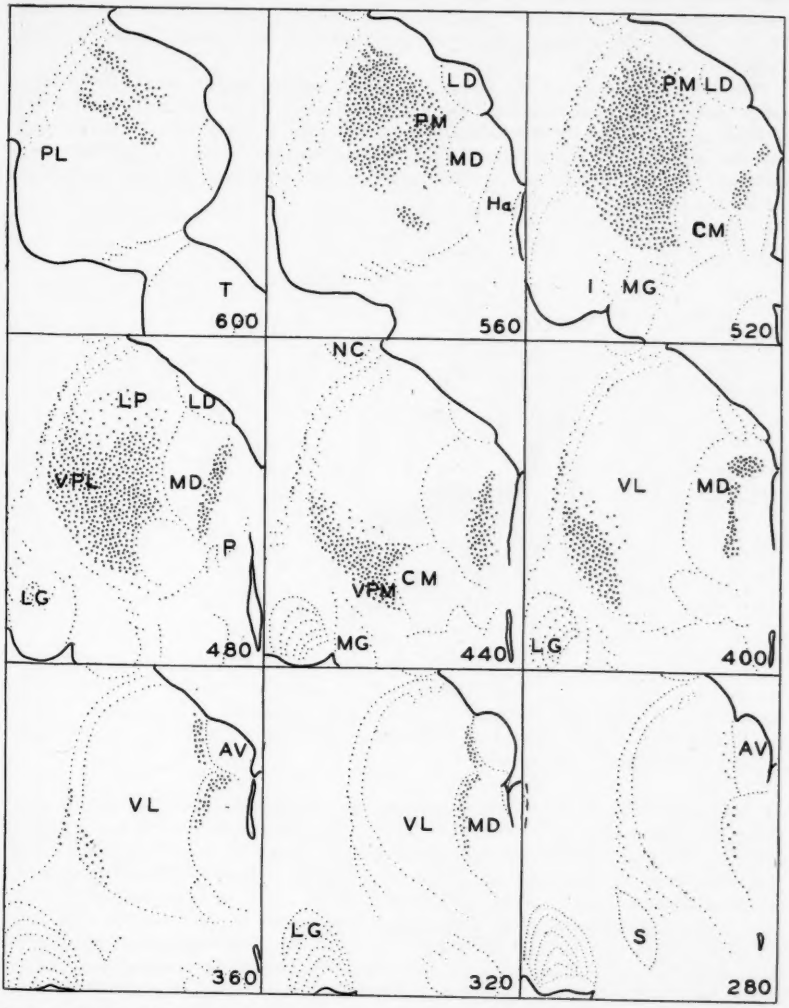

Fig. 20 (Exp. 5, chimpanzee "Carrie Nation"). Representative sections of the left thalamus to show the site and extent of the retrograde cell degeneration.

parieto-occipital sulcus, the anterior margin of which is ablated. The lesion follows this sulcus to within 2 cm. of its inferior tip. The inferior margin of the lesion cuts across the angular and supramarginal gyri and the sylvian fissure to the inferior tip of the central sulcus. The entire angular and supramarginal gyri have been removed

leaving intact all but the posterior part of the first temporal convolution. The lesion measures 2 cm. along the midline, and 2·7 cm. across its base inferiorly and extends along the central sulcus for a distance of 4·6 cm. The base of the ablation is covered with haemorrhagic infiltrated white matter, in which structural details cannot be distinguished. It is depressed 1 cm. below the normal contour at its deepest point. At the inferior end of the central sulcus the lesion is not as deep, measuring only 5 cm. (Fig. 21).

The blocks removed at the second operation were sewed in place. In order to study better the cytoarchitecture the block containing the lesion was divided by a sagittal cut just superior to the inferior bend of the central sulcus. Both blocks were cut serially. The first is from the upper portion of the lesion (*A*), and the second the lower (*B*). From both, every 25th section has been stained, and the sections are cut at 25μ (Fig. 22).

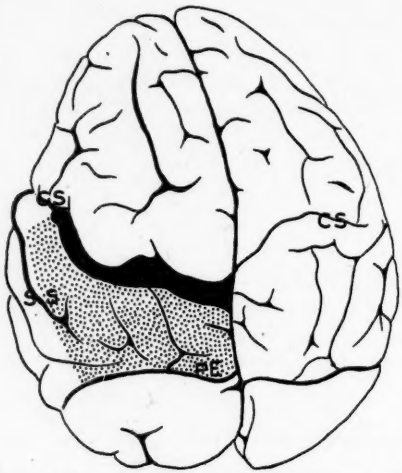

Fig. 21 (Exp. 6, chimpanzee "Maggie"). The cerebral hemispheres of chimpanzee "Maggie" showing (in black) the ablated area and (stippled) the area removed at the second operation from which the animal did not survive.

A careful examination of these sections shows that the anterior margin of the lesion near the midline is bordered by the extreme anterior margin of area 3, slightly inferiorly the zone of area 3 between the lesion and area 4 becomes wider. The margin continues along the posterior lip of the central sulcus, bounded by a strip of area 3 on the anterior wall of the sulcus and in the trough. Near the beginning of the "arm area", where the central sulcus sweeps anteriorly, more of the posterior wall of the sulcus is spared and both areas 3 and 1 are present. The lesion continues in this fashion to within 2 cm. of the tip of the central fissure where it terminates. In this region the lesion is bounded by cortex of area 1. In only one point is area 4 infringed upon, just above the anterior bend of the central sulcus where a very superficial lesion damages only its outer two layers (Fig. 22).

The posterior margin of the lesion must be determined by the cortex remaining in the depth of the lesion and the specimens from the second operation. From an examination of these, the inferior margin of the lesion appears to be in area 7. Although a small fragment of cortex is still present about the trough of the post-central sulcus, it does not appear to contain area 2 cortex. There is, however, a small

fragment of area 2 cortex present in the upper part of the second operative specimen. This, however, extends over only a few millimetres. Therefore, the lesion of the primary operation has removed most of areas 1 and 2, and a small part of areas 3, 5 and 7 and has not damaged the cortex of the precentral convolution.

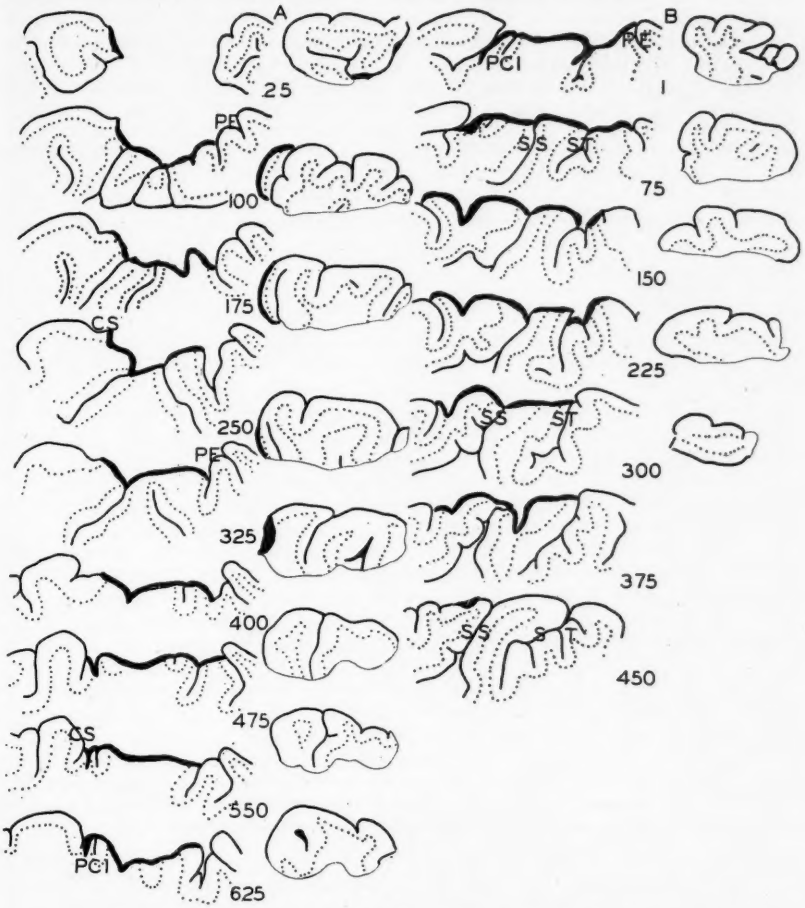

Fig. 22 (Exp. 6, chimpanzee "Maggie"). The serial sections of the entire parietal lobe (left) and of the tissue ablated at the second operation (right). The first two columns are the sections from the superior portions of the parietal lobe, and the second two from the inferior portion.

The thalamus. The thalamus has been sectioned serially in a sagittal plane. An examination of these sections shows that the degeneration is limited to the nucleus ventralis posterior which is almost completely degenerated. The suprajacent lateral nucleus does not seem to be involved by the degeneration, only two suspicious areas being present. The one is slightly lateral to the external medullary lamina in the middle portion of the nucleus lateralis posterior, and the other is situated medially

near the anterior extremity of the nucleus lateralis posterior. It is possible that these areas are not true degeneration but either fibre tracts, or due to a small protrusion of the nucleus ventralis posterior. A small portion of the anterior layer of the nucleus ventralis posterior remains relatively normal and here scattered large cells characteristic of this nucleus are present. The most medial portion of the nucleus ventralis posteromedialis subjacent to the nucleus centrum medianum is relatively spared, although there is an area of degeneration even in this part. Areas of this portion of the nucleus ventralis posterior present a normal picture. The nucleus ventralis lateralis appears normal as does also the pulvinar, the geniculate bodies and the nucleus medialis dorsalis (Fig. 23).

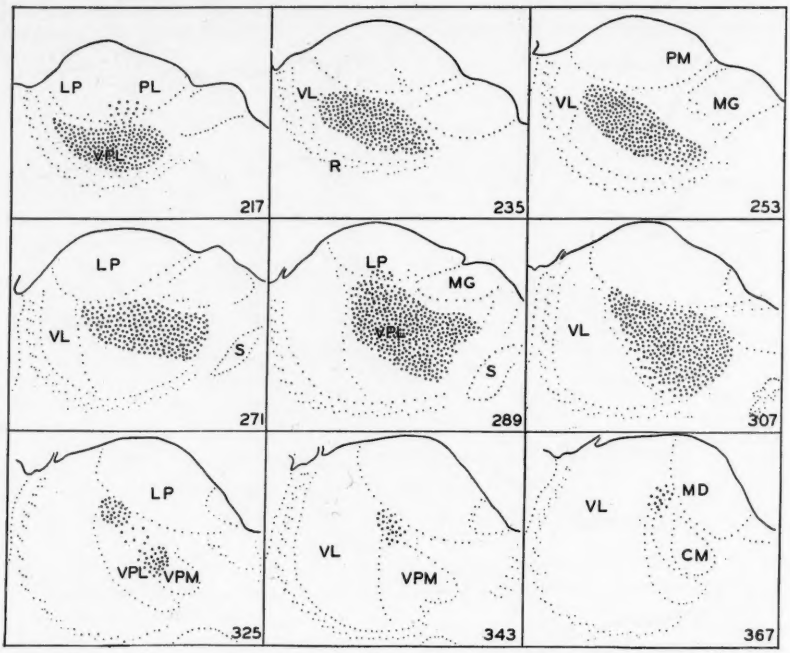

Fig. 23 (Exp. 6, chimpanzee "Maggie"). Serial sagittal sections of the left thalamus of chimpanzee "Maggie" to show the extent of the retrograde cell degeneration. The sections at the left are lateral, those at the right medial.

The lesion then, which has removed all except the inferior 2 cm. of the post-central convolution, has given rise to severe degeneration in the nucleus ventralis posterior, and practically no degeneration in any other nucleus.

The findings in this case demonstrate that the nucleus ventralis posterior projects almost entirely to the postcentral convolution, and that other nuclei do not participate in this radiation.

In the macaque monkey, a precise spatial organization of the projection to the posterior convolution has been demonstrated. That such an arrangement probably also holds for the chimpanzee is shown by the following experiment.

Exp. 7. *Successive left hemidecortication, and ablation of right arm motor and sensory area; linear degeneration in the nuclei ventralis posterior and ventralis lateralis. Weight* 21·0 *kg.* "*Mary.*"

The subject of this experiment was an immature female chimpanzee. On 11 September 1935 under sodium amytal anaesthesia Dr Fulton reflected a large left osteoplastic flap and extirpated the entire cerebral cortex of the left hemisphere leaving only a small fragment of the cortex on the inferior surface of the frontal lobe, and the hippocampal gyrus. The animal had an uneventful convalescence. On 9 October 1935 under sodium amytal anaesthesia a right osteoplastic craniotomy was performed and the cortex of the pre- and postcentral convolutions in the arm area removed. The convalescence was also uneventful, and the animal was sacrificed 15 November 1935. The vascular system was perfused with saline followed by alcohol.

Anatomical examination

Right cerebral hemisphere. The cortex of the right cerebral hemisphere appears quite normal except for the ablation along the middle of the central sulcus. This rectangular shaped lesion measures 1·5 cm. in length along the sulcus and 1 cm. in width. It lies in the concavity of the central sulcus and reaches from the inferior end of the superior precentral sulcus to the inferior extremity of the postcentral sulcus. The supero-anterior corner lies in the precentral convolution, while the remainder occupies the postcentral convolution. Serial sections of the cortex confirm the site of the lesion, and show that it has damaged only a very small part of the precentral convolution. The injury to the cortex of the precentral convolution is confined to area 4, at no point infringing upon area 6 for more than a millimetre. The greater part of the damage is in the postcentral gyrus which in the middle of the lesion is entirely removed. At one point even the cortex in the trough of the central sulcus has been removed. Inferiorly the lesion follows the postcentral sulcus and the cortex of areas 3 and 1 along the central sulcus is undamaged, area 2 suffering the greatest injury (Fig. 24).

Right thalamus. A careful examination of serial sections of the right thalamus reveals that the degeneration is confined to the nuclei ventralis posterior and ventralis lateralis. Posteriorly it begins just rostral to the caudal extremity of the nucleus centrum medianum in the medial portion of the nucleus ventralis posterior, bordering the anterior pole of the nucleus pulvinaris medialis. Anteriorly the area of degeneration rapidly enlarges to a sickle-shaped zone within the nucleus ventralis posterior. In the involved area practically no normal neurons are present. The degeneration maintains relatively the same shape and position throughout the entire extent of the nucleus ventralis posterior. Caudally it extends slightly into the nucleus lateralis posterior but the degeneration in this nucleus is more diffuse than in the subjacent one. At the oral extremity of the nucleus ventralis posterior the degeneration continues without interruption in the nucleus ventralis lateralis. Although maintaining the same relative position as in the nucleus ventralis posterior, it is more scattered and less well defined. It becomes less distinct and disappears near the middle of the nucleus ventralis lateralis (Fig. 25).

The arrangement of the thalamic projection to the postcentral convolution is further demonstrated by another experiment.

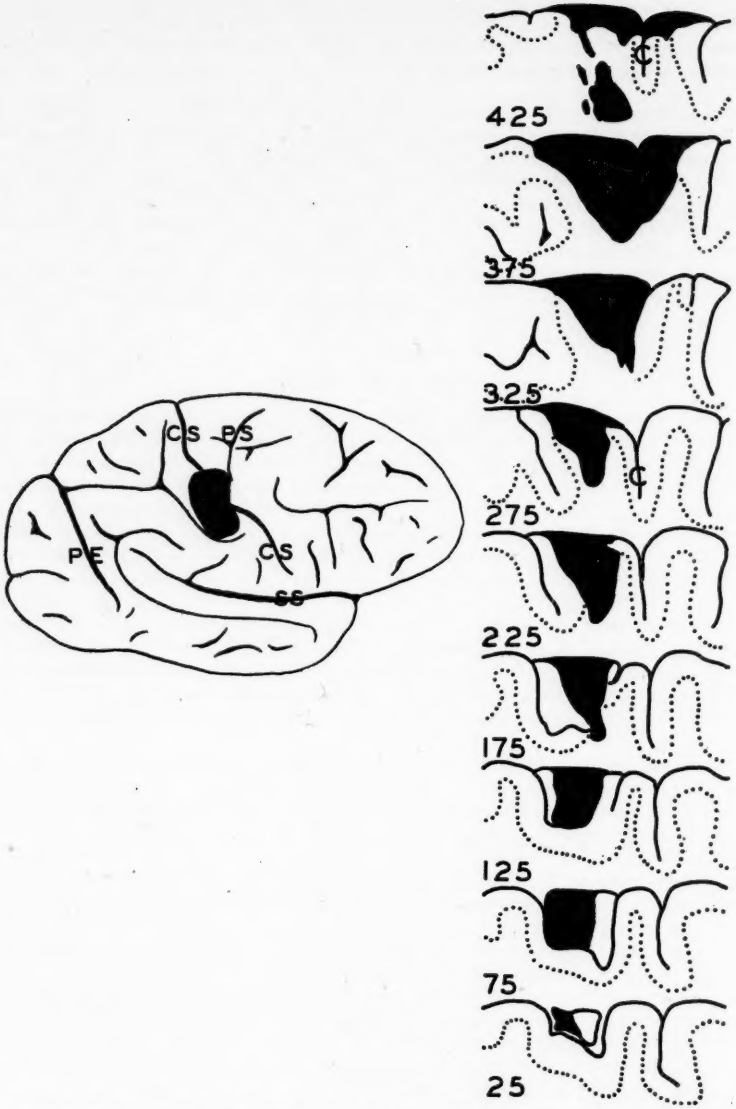

Fig. 24 (Exp. 7, chimpanzee "Mary"). The right cerebral hemisphere of chimpanzee "Mary", and at the right serial sections of the lesion, cut at right angles to the central sulcus.

Exp. 8. *Ablation of the inferior two-thirds of the right postcentral convolution and the occipital operculum; severe degeneration in the nucleus ventralis posterior and a small zone in the nucleus pulvinaris lateralis. Weight* 11·3 *kg.* "*Snookums.*"

The subject of this experiment was a timid, temperamental young female chimpanzee in excellent condition except for a desquamating dermatitis over the head and neck. On 19 February 1936 a right osteoplastic craniotomy was performed.

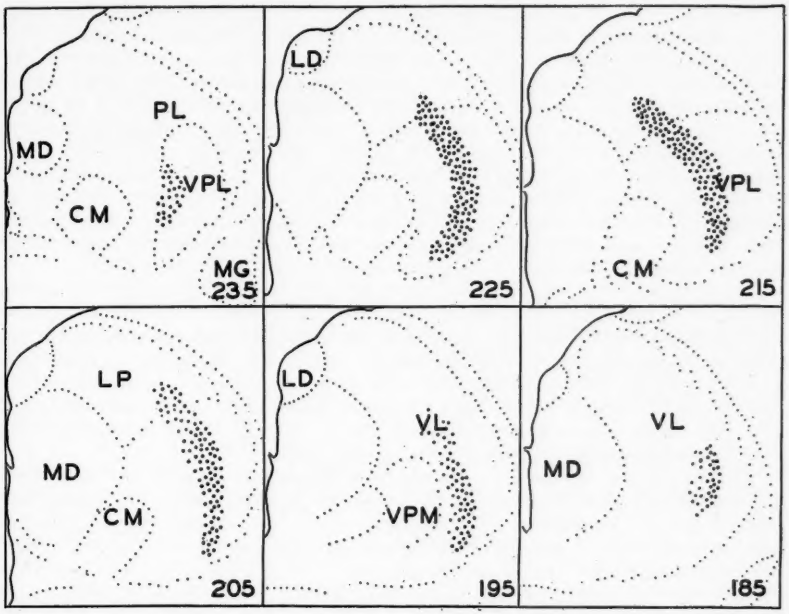

Fig. 25 (Exp. 7, chimpanzee "Mary"). Representative serial sections of the right thalamus of chimpanzee "Mary" to show the position and extent of the retrograde cell degeneration.

The inferior half of the postcentral convolution and the occipital operculum juxtaposed to the external parieto-occipital fissure were removed. 2 April 1936 a left osteoplastic craniotomy was performed exposing the occipital and posterior temporal regions. The left occipital lobe posterior to the parieto-occipital fissure was removed in one piece. The sylvian fissure was opened and with a brain spatula the cortex lying upon a small ridge on the superior surface of the first temporal gyrus was removed by subpial dissection. On 28 May 1936 the animal was sacrificed.

Anatomical examination

Right cerebral hemisphere. The right cerebral hemisphere is the site of two lesions, one occupying the inferior two-thirds of the postcentral convolution, and the second the anterior portion of the lateral surface of the occipital lobe. The first lesion is 3·5 cm. in length and 0·6 cm. in width. It is bounded in front by the anterior wall of the central sulcus and behind by the posterior wall of the inferior postcentral

sulcus. At the inferior extremity of the postcentral convolution 1·5 cm. of normal cortex is present (Fig. 26).

An examination of the serial sections of the blocks from this lesion shows that the ablation has removed almost all the lower two-thirds of the postcentral convolution. The lesion is bounded anteriorly by area 3 cortex. The posterior margin of the lesion except at its extremities is formed by the cortex of the parietal lobules (areas 5 and 7). There is no evidence of areas 1 or 2 posteriorly except inferiorly near the tip of the central sulcus where the lesion is bounded by area 2 cortex. Beneath this extending inferiorly to the sylvian fissure is cortex of types 3 and 1. The second lesion is an ablation of the occipital operculum paralleling the parieto-occipital sulcus. It begins 1 cm. from the midline as a lesion 0·6–0·7 cm. in width tapering inferiorly to its termination just above the lateral tip of the sulcus occipitalis diagonalis.

Serial sections of the occipital lobe confirm the gross location of the lesion. Its posterior margin borders striate cortex. The anterior margin on the posterior wall of the external parieto-occipital sulcus is bounded mainly by cortex of type 18 and in a few places by type 19. In the lower part of the lesion there is slight involvement of the annectant cortex of the posterior parietal lobe, but the greater part of the apparent damage is the result of accidental haemorrhage at the time of sacrifice, considerable fresh blood being present in the parieto-occipital sulcus and a small amount in the tissues of the cortex anterior to the external parieto-occipital sulcus. There is, however, a small old lesion of this cortex. In no place does the lesion extend into the white matter for more than 1–2 mm. (Fig. 27).

Right thalamus. A careful examination of serial sections of the right thalamus shows severe degeneration in the nucleus ventralis posterior and a small area of cellular change in the nucleus pulvinaris lateralis. The posterior portion of the pulvinar is entirely normal but just caudal to the junction of the pulvinar and mesencephalon a small localized area of degeneration is seen above the cortico-mesencephalic tracts in the nucleus pulvinaris lateralis. This area may be traced anteriorly in the same relative position to the level of the caudal extremity of the lateral geniculate body where it disperses and disappears (Fig. 28).

Just caudal to the nucleus ventralis posterior a scattering of retrograde cell degeneration is seen in the nucleus pulvinaris lateralis, slightly dorsal to the previous zone. A few sections anteriorly the posterior pole of the nucleus ventralis posterior appears along the external medullary lamina, and the degenerated area, now larger and better defined, involves its medial portion. A second and smaller area of cellular change—somewhat more diffuse—is present still more medially in the nucleus. At the caudal pole of the nucleus centrum medianum the degeneration becomes more pronounced to involve the entire medial two-thirds of the nucleus ventralis posterior. A zone of the characteristic large cells of the latter nucleus lies along the external medullary lamina. The degenerated area does not extend superiorly into the nucleus lateralis posterior, but appears to be entirely confined to the nucleus ventralis posterior. The degeneration anteriorly maintains the same relative position in the medial part of this nucleus completely sparing the paralamellar portion. A narrow zone of normal neurons, three or four cells in width, lies along the margin of the nucleus ventralis posterior bordering the rostral pole of the nucleus centrum medianum. Slightly anteriorly in this position the transition to the nucleus ventralis lateralis occurs. Laterally the nucleus ventralis posterior extends rostrally, the zone of degeneration following its medial border. Shortly rostral to the oral extremity of the nucleus centrum medianum the degeneration becomes less definite and just caudal to the oral extremity of the nucleus ventralis posterior it disappears. The nucleus ventralis lateralis appears normal.

The analysis of the origin of the degenerations in this experiment presents little difficulty. The changes in the nucleus ventralis posterior are the result

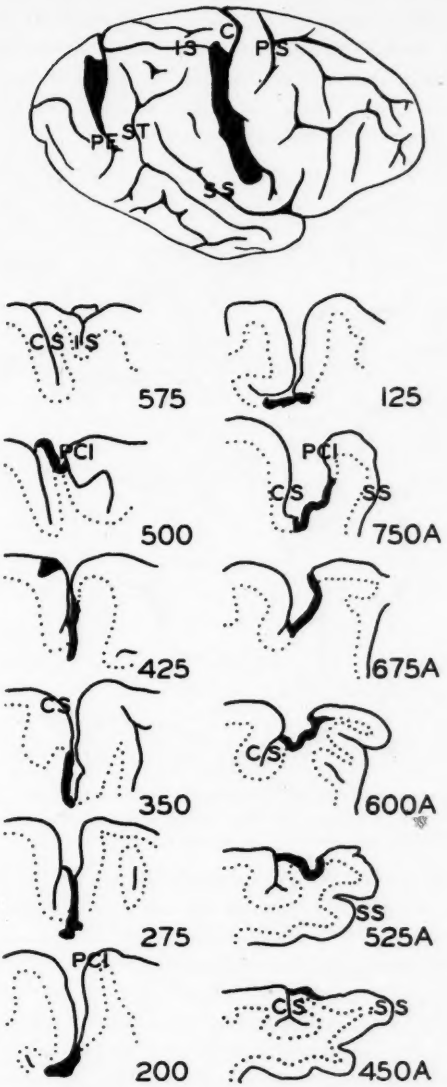

Fig. 26 (Exp. 8, chimpanzee "Snookums"). The right cerebral hemisphere of chimpanzee "Snookums" and below serial sections of the lesion of the postcentral convolution cut at right angles to the central sulcus. The sections of the columns to the left are from the upper portion of the lesion.

of the ablation of the inferior portion of the postcentral convolution, since Exp. 6 has conclusively shown that this is the projection area of the ventral posterior nucleus. The small zone of degeneration in the nucleus pulvinaris lateralis must then be due to the lesion of the occipital operculum. Whether it is the result of the injury to the parastriate areas or to the posterior parietal cortex cannot be determined from this experiment.

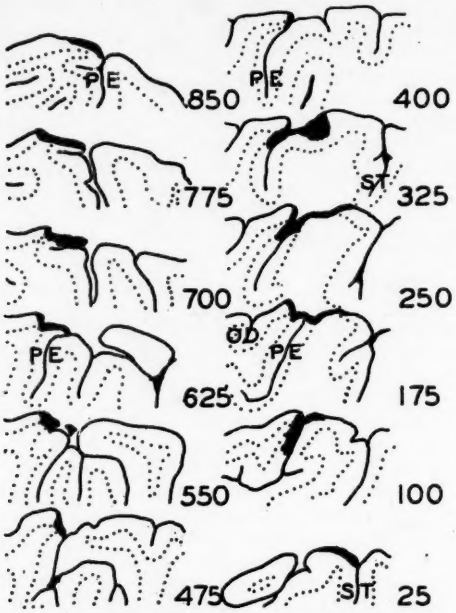

Fig. 27 (Exp. 8, chimpanzee "Snookums"). Serial sections of the lesion of the occipital operculum. The sections from the upper portion of the lesion are in the column at the left. The sections are cut at right angles to the external parieto-occipital fissure.

The findings in the last two experiments are sufficiently clear to state that the organization of the projection of the nucleus ventralis posterior to the postcentral convolution in the chimpanzee is the same as that in the macque monkey. The most medial portion of the nucleus sends its fibres to the inferior part of the postcentral gyrus, the lateral part to the cortex abutting the sagittal fissure and the intermediate to the middle portion of the postcentral convolution. It is thus apparent that the organization of the radiations to the pre- and postcentral convolutions closely parallel each other.

(3) *Temporal and occipital lobes*

While the fibre connexions to the frontal and parietal regions have been studied in detail by previous investigators, the projections to the occipital and temporal lobes are little mentioned. Even the recent studies are far from in

agreement as to the detailed arrangement of the thalamic projection to these parts. Although visual and auditory radiations are the predominant efferent fibres to these regions other thalamic projections are present.

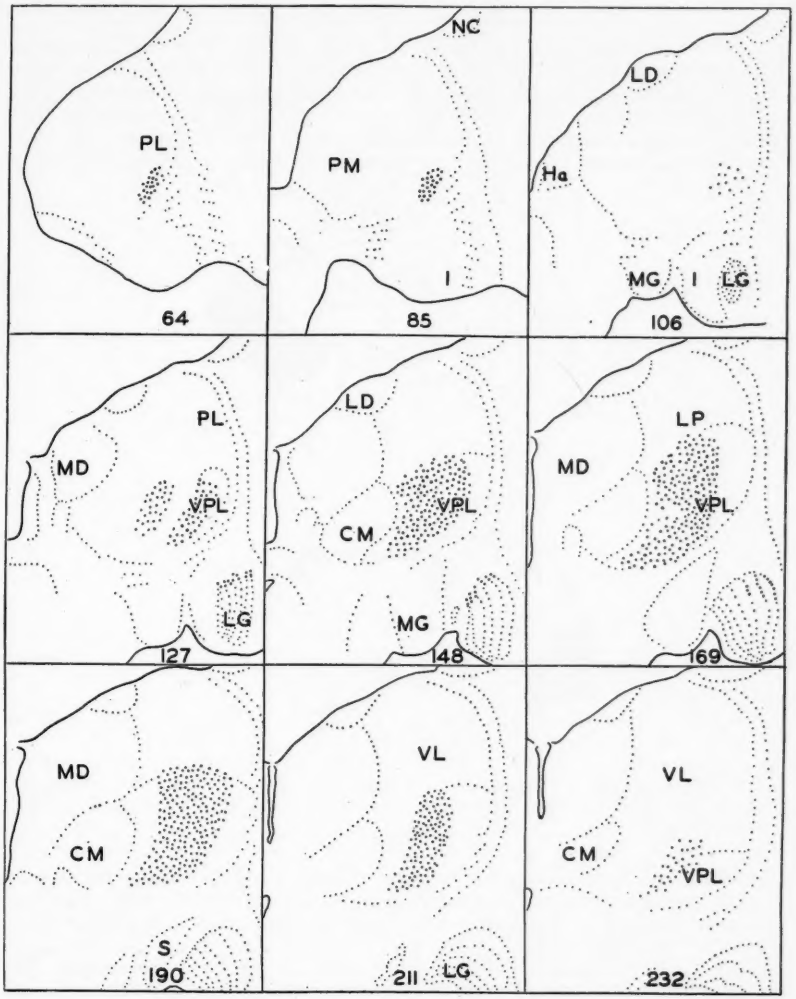

Fig. 28 (Exp. 8, chimpanzee "Snookums"). Representative sections of the right thalamus of chimpanzee "Snookums" to show the extent and position of the retrograde cell degeneration.

Exp. 8 (*cont.*). *Left occipital lobectomy and small lesions of the superior surface of the first temporal convolution; severe degeneration in the nucleus pulvinaris inferior and the lateral and medial geniculate bodies.* "*Snookums.*"

Anatomical examination

Left cerebral hemisphere. Two lesions are visible in the left cerebral hemisphere. Immediately below the inferior tip of the inferior postcentral sulcus a linear injury 0·8 cm. in length lies along the superior margin of the first temporal convolution but does not involve the cortex on the convexity of that gyrus. This lesion has been described in a previous paper (Walker & Fulton, 1938). The occipital lobe posterior to the parieto-occipital fissure has been ablated. The cortex on the anterior wall of

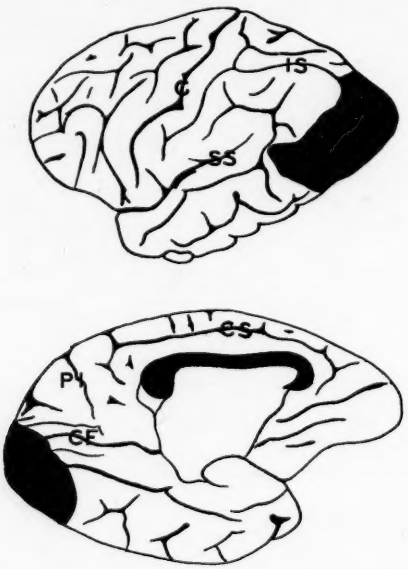

Fig. 29 (Exp. 8, chimpanzee "Snookums"). The left hemisphere of chimpanzee "Snookums" to show the site of the cortical ablations. Above, lateral surface of the hemisphere, below, medial surface.

this sulcus has been damaged in varying degrees. Inferiorly a superficial lesion extends almost to the posterior margin of the superior temporal sulcus. Superiorly the annectant gyri are damaged but the superior parietal lobule is uninjured. The cuneus on the medial surface of the hemisphere is intact except for the ablated superior lip of the calcarine fissure. On the inferior surface of the occipital lobe the damage is more extensive than on the medial surface of the hemisphere (Fig. 29).

Serial sections of the occipital lobe show that the striate cortex of the entire lateral surface has been ablated, but along the anterior margin of the calcarine fissure within its depth a small strip is preserved. Somewhat more of the striate cortex is present on the superior lip of the calcarine fissure than on the inferior. Area 18 bounds the lesion about the depths of the parieto-occipital fissure, but the greater part is bounded by cortex of area 19. The lesion on the lateral surface of the hemisphere

has this type of cortex on its margins except in the angular gyrus where area 7 bounds it (Fig. 30).

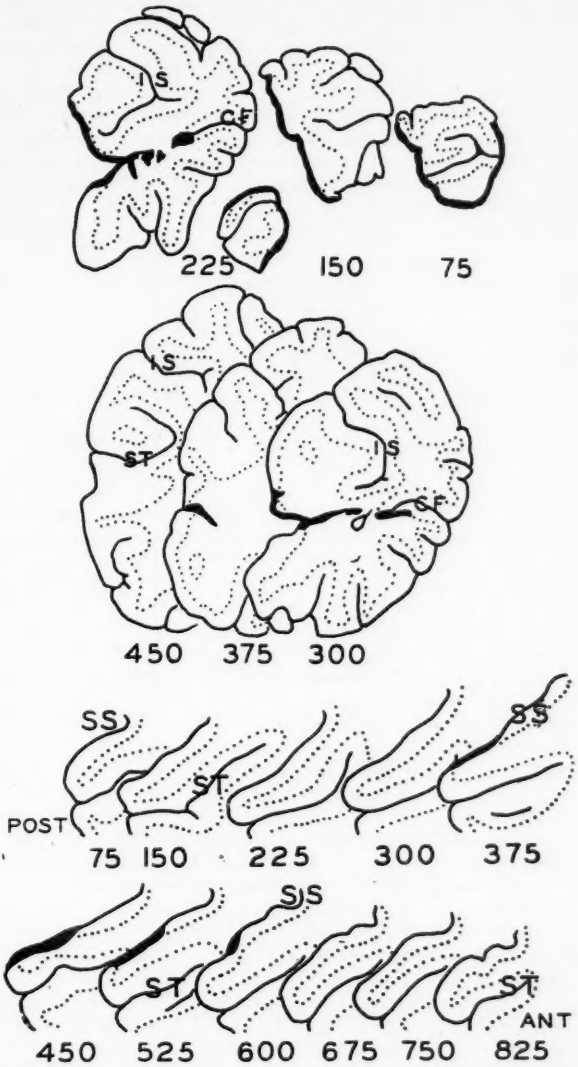

Fig. 30 (Exp. 8, chimpanzee "Snookums"). Serial sections of the lesions of the left cerebral hemispheres, above of the occipital lobe, and below of the first temporal convolution.

The left thalamus. The degeneration within the thalamus proper is confined to the pulvinar. That within the geniculate bodies has been discussed in a previous paper. The pole of the pulvinar shows no evidence of cellular alteration, but a short

distance caudal to the junction of the pulvinar and mesencephalon, marked retrograde cell degeneration is present in the lateral portion of the pulvinar (nucleus pulvinar lateralis). This degeneration, at first spotty and irregular, coalesces in the ventrolateral portion of the pulvinar, which anteriorly becomes the nucleus pulvinaris inferior. In this nucleus the degeneration is severe except for a small strip of normal cells bordering its inferior and medial margin. The degeneration also involves the ventrolateral portion of the nucleus pulvinaris lateralis just above the cortico-mesencephalic tracts. The degeneration continues in the same position to the anterior pole of the nucleus pulvinaris inferior. The degeneration in the most ventral and lateral portion of the nucleus pulvinaris lateralis may be followed to the same level where it becomes less pronounced and gradually disappears before the advent of the nucleus ventralis posterior (Fig. 31).

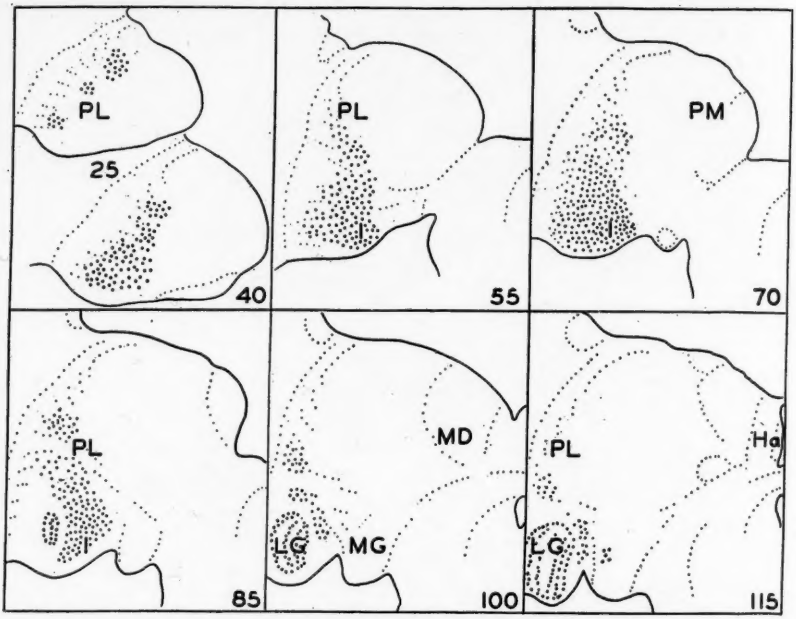

Fig. 31 (Exp. 8, chimpanzee "Snookums"). Serial sections of the left thalamus to show the extent and situation of the retrograde cell degeneration.

It is evident from this experiment that the inferior nucleus of the pulvinar sends its fibres to the inferior temporo-occipital region. Apparently none of the other thalamic nuclei project to the occipital lobe.

In another experiment a large lesion was made in the temporal lobe.

Exp. 9. *Ablation of the right first and second temporal convolutions; severe atrophy in the medial and lateral geniculate bodies and in the nucleus pulvinaris inferior. Weight* 22·1 *kg.* "*Ghandi.*"

The subject of this experiment was an immature male chimpanzee. At the first operation, 8 November 1935, lesions were made in the left motor "leg area" and a

superficial one in the left supramarginal gyrus. On 4 December 1935 under sodium amytal anaesthesia a low right craniotomy was performed. The first and second temporal convolutions from near the tip of the temporal lobe to the inferior margin of the angular and supramarginal gyri were removed en bloc. The convalescence was uneventful. At subsequent operations further lesions were made in the left cerebral hemisphere. On 31 March 1936 the left superior peduncle was sectioned. No lesion was made in any part of the cerebral cortex at this time. The animal was sacrificed 3 April 1936.

Anatomical examination

All lesions were sectioned serially so that the precise extent of the injuries could be accurately ascertained.

Right cerebral hemisphere. The right cerebral hemisphere is the site of a lesion occupying the first and second temporal convolutions extending from near the tip of the temporal lobe posteriorly for 3·0 cm. and inferiorly from the sylvian fissure 1·4 cm. It is bounded by the superior lip of the sylvian fissure and the cortex of the island of Reil both of which appear undamaged. Posteriorly a part of the superior temporal convolution remains and the margin of the lesion slopes inferiorly to the middle temporal sulcus. This sulcus forms the inferior margin of the ablation in most of its extent. Anteriorly a small part of the the tip of the temporal lobe remains. The anterior two-thirds of the first and second temporal convolutions have been largely ablated (Fig. 32).

Serial sections of the temporal lobe confirm the gross location of the lesion. The superior margin of the lesion follows the lower angle of the sylvian fissure from almost the tip of the temporal lobe to the posterior margin of the insula sparing entirely the cortex of the latter. At this point on the inferior lip of the sylvian sulcus, cortex of the granular type (koniocortex) bounds the lesion. The latter rapidly becomes superficial, completely sparing the posterior and medial portion of the auditory koniocortex. The inferior border of the lesion along the inferior temporal convolution is bounded by area 20 cortex. Near the posterior extremity of the lesion the cortex appears like that of area 21. The lesion extends deeply into the substance of the temporal lobe but a narrow margin of white matter surrounds the lateral surface of the inferior horn of the lateral ventricle. There is no evidence of direct damage to the striate cortex in any part of the lesion. In fact there is no striate cortex in any of the sections of the cortex cut well posterior to the lesion.

Right thalamus. A careful examination of serial sections of the thalamus shows that the degeneration is entirely confined to the pulvinar and the geniculate bodies. The sections have been cut on a semi-horizontal plane so that the superior parts of the section contain thalamic elements which are more anterior than those in the inferior part of the section.

Along the inferior margin of the posterior pole of the pulvinar a zone of marked gliosis with slight cellular degeneration is present. In anterior sections it rapidly enlarges and extends medially almost across the pulvinar—well into the nucleus pulvinaris medialis, but remains confined to the inferior portion of the thalamus. Still more rostrally the degeneration becomes particularly marked in the nucleus pulvinaris inferior but in spite of the intense gliosis a few normal cells remain. The changes, gradually diminishing in intensity, may be traced to the anterior extremity of the nucleus pulvinaris inferior. There is no degeneration in any part of the nucleus ventralis posterior, nor in the adjacent parts of the lateral nuclear mass (Fig. 33).

The changes in the thalamus in this case are practically identical with those in the left thalamus of Exp. 8. It is therefore very probable that the degeneration in the nucleus pulvinaris inferior in this case is due to interruption

of fibre tracts passing through the temporal lobe to the inferior temporo-occipital region. One must conclude that the thalamic projection to the temporal lobe is slight or lacking entirely.

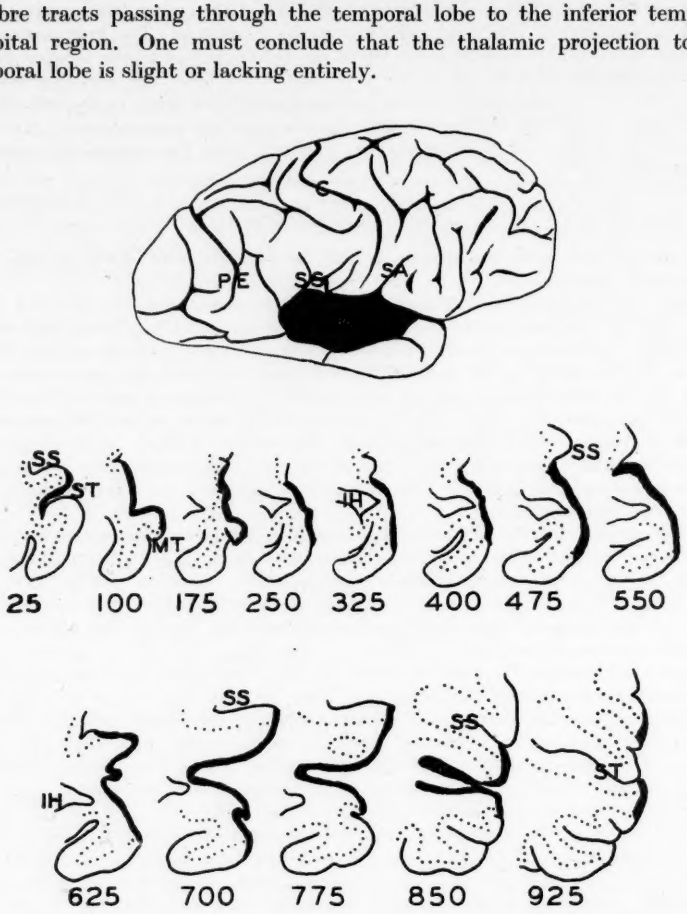

Fig. 32 (Exp. 9, chimpanzee "Ghandi"). The right hemisphere of chimpanzee "Ghandi" to show the site and extent of the cortical lesion. The serial sections of the lesion are cut at right angles to the sylvian fissure.

III. PROJECTION OF THE THALAMIC NUCLEI UPON THE CEREBRAL CORTEX

The general topical projection of the thalamus has been discussed in some detail, but the connexions of the individual nuclei have not previously been analysed. With the above data at hand the principal relations become fully intelligible.

Anterior group of nuclei. Although the anterior nuclei of the chimpanzee degenerate following complete hemidecortication in none of the present experi-

ments have they shown changes. It is therefore apparent that they project to some portion of the cerebral cortex which was not damaged in the above experiments. Two possible regions are present, (i) the posterior part of the cingular gyrus, and (ii) the inferior surface of the temporal lobe. The present study does not present evidence in favour of either of these areas. However, experimental studies in lower primates suggest that a projection to the posterior part of the cingular gyrus is the most likely (Walker, 1938*c*).

Although Déjerine (1901), Sachs (1909) and Flechsig (1876) denied a cortical projection from the anterior nuclei, the majority of investigators have accepted this thesis. However, the site of termination of the projection is

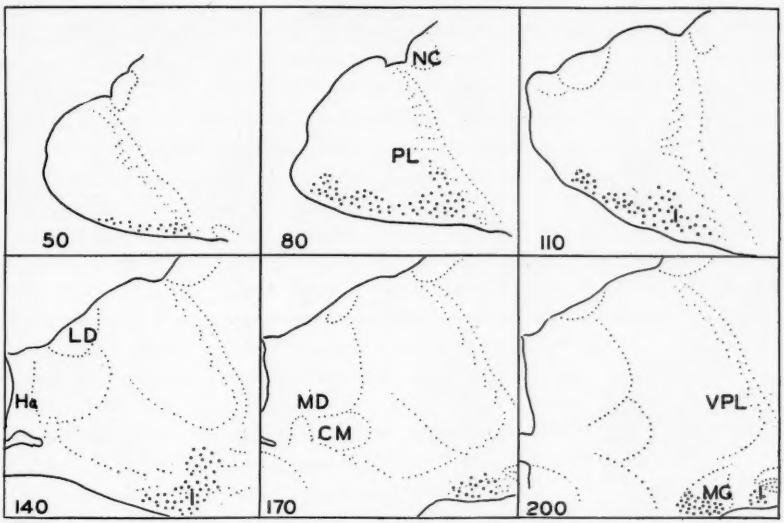

Fig. 33 (Exp. 9, chimpanzee "Ghandi"). Serial sections of the thalamus of chimpanzee "Ghandi" to show the extent of the retrograde cell degeneration.

obscure. Monakow (1895) was one of the first to describe connexions to the frontal lobe. Others (Roussy, 1907; Mingazzini, 1913; and Minkowski 1923–4) have come to similar conclusions. In cats and rats Le Gros Clark & Boggon (1932) traced Marchi degeneration from lesions in the anterior nuclei to the cingular gyrus. In one of their monkeys (*L*) they describe mild cellular changes in the anterior nuclei as the result of a lesion of the anterior portion of the cingular gyrus. Using the method of retrograde cell degeneration Waller came to a similar conclusion. Walker (1936) stated that in monkeys, the antero-medial nucleus showed degeneration following lesions of the inferior and medial surface of the frontal lobe. He concluded that the other anterior nuclei project upon the posterior part of the cingular gyrus. In his recent monograph (Walker, 1938*c*) he gives support for this contention, since, following a lesion

of the posterior part of the cingular gyrus, degeneration was found in the nucleus anteroventralis.

Nuclei of the midline. In none of the present experiments has degeneration been observed in the midline group of nuclei. Nor were changes present in that group following complete hemidecortication; hence it is clear that these

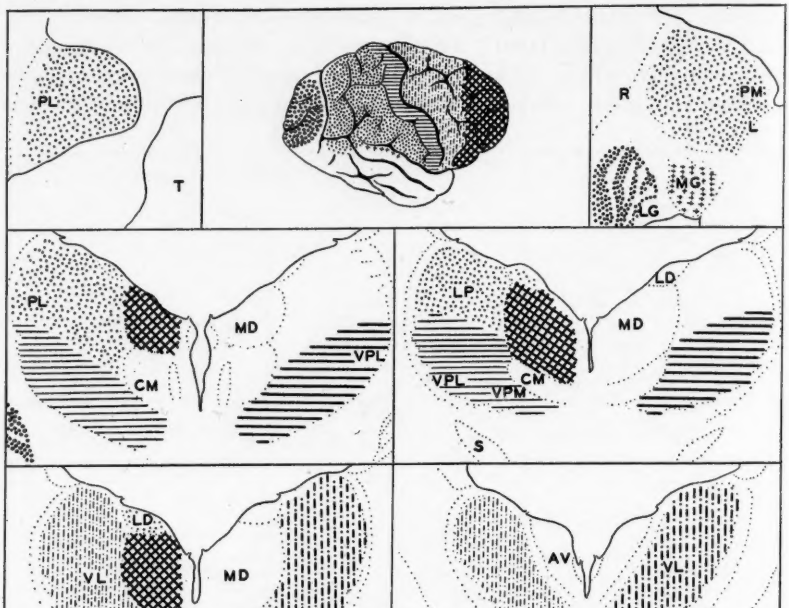

Fig. 34. Schema showing the thalamocortical projection. The thalamic nuclei project to the similarly marked parts of the cerebral cortex. In the right thalamus the terminations of the ascending somatic tracts are indicated (lemnisci and spinal thalamic tracts by heavy horizontal lines and brachium conjunctivum by interrupted heavy dots and dashes).

nuclei do not project to the cerebral cortex. The present experiments give no clue concerning their connexions, but previous investigators have described numerous subcortical relationships.

Many authors have described finely myelinated or non-myelinated fibres swinging about the third ventricle to the hypothalamic region. Allen (1923, rat) and Rioch (1929, dog and cat) have studied these pathways in detail. Roussy & Mosinger (1934) have divided them into five groups.

1. Internal periventricular thalamo-hypothalamic tract from the midline nuclei.

2. External periventricular thalamo-hypothalamic tract from the medial thalamic nucleus (magnocellular part).
3. Internal lamellothalamic tract.
4. External lamellothalamic tract.
5. Fibres from the commissure of Ganser.

Huber & Crosby (1929) discuss these connexions in their summary of the diencephalic relations. In the macaque Walker (1936) found degeneration in the midline nuclei as the result of a lesion in the supraoptic region.

The medial nuclei. This group consists of the large medial nucleus and the intralaminar nuclei. The first of these degenerates in consequence of lesions of the frontal tip; the others have shown no degeneration in any of the above experiments or in the hemidecorticated chimpanzee. The projection of the nucleus medialis dorsalis has been demonstrated to be to the granular frontal area. In every case in which this cortex has been injured, degeneration has been present in the main medial nucleus of the thalamus.[1] The projection from this nucleus to the cortex appears to have a precise organization, for the degenerations from variously placed lesions are quite distinct. The exact organization of this projection, however, cannot be determined for the chimpanzee from the evidence available. Certain inferences, however, are allowable. The paralamellar portion (lateral) of the nucleus seems to project to the superior part of the granular cortex (Exp. 3), and the inferior and medial portions of the nucleus to the cortex on the orbital edge of the frontal lobe. The findings in these experiments are compatible with the hypothesis that anteroposterior planes of the nucleus medialis dorsalis project upon points of the cortex.

The intralaminar nuclei in the above series have shown no definite degeneration. In the thalamus of the hemidecorticated chimpanzee a slight decrease in cells has been noted in some of these nuclei but such changes have been minimal. No evidence regarding the projections of these nuclei can be adduced from the present investigation. Studies on lower primates and carnivora suggest that their main connexions are to the hypothalamus and basal ganglia.

Normal preparations stained for myelin suggest such subcortical connexions (Allen, 1923; Rioch, 1929; and Huber & Crosby, 1929). Walker (1936) has described degeneration in certain of the intralaminar nuclei as the result of a lesion of the supraoptic region. Extensive degeneration was noted in the intralaminar nuclei, also, in particular the nucleus centrum medianum in an animal in which the corpus striatum had been damaged in the process of hemidecortication. Recently Papez & Rundles (1937) have discussed the

[1] Practically all investigators since the monumental studies of Monakow (1895) have found that the main medial nucleus of the thalamus projected to the cerebral cortex. Most workers agree that it projects to the frontal lobe. Sager (1933) on incomplete evidence denied such connexions and Poliak (1932) does not describe them, probably because his lesions did not damage the radiation from the nucleus medialis dorsalis which passes inferiorly in the rostral portion of the internal medullary lamina.

connexions of this nucleus. They consider that its fibres pass to several subcortical structures including the striatum.

Lateral nuclear mass. The component parts of this group project to quite discrete areas. In none of the above experiments has the nucleus ventralis anterior shown significant degeneration. This part of the lateral nuclear mass, as studies on lower primates show, probably sends its fibres to the corpus striatum. Many observers have described myelinated fibres from the thalamus crossing through the ansa lenticularis to the corpus striatum. In the monkey Walker has observed degeneration of this nucleus in a hemidecorticated animal in which the corpus striatum was severely damaged.

From the above experiments it is apparent that the nucleus ventralis lateralis, as in the monkey, projects to the motor and premotor areas exclusively with a precise arrangement. In the macaque monkey it has been demonstrated that area 4 receives the greater part of the thalamic projection from this nucleus. The findings in Exp. 3 suggest that this also applies to the chimpanzee. The projection to area 6 originates from a slightly more anterior part of the nucleus than that for area 4. The arrangement of the projection from this nucleus has been referred to previously. The medial portion of the nucleus projects to the inferior motor and premotor cortex; the paralamellar part to areas 4 and 6 near the midline; and the intermediate part of the nucleus to the intervening cortex. There is then a precisely localized topographical thalamic projection to the precentral convolution. The significance of this connexion to the motor areas of the cerebral cortex, as originally pointed out, lies in the fact that the brachium conjunctivum terminates in the nucleus ventralis lateralis (Walker, 1936). By this arrangement, then, a mechanism is created whereby the cerebellum may maintain a co-ordinating influence upon the activity of the motor areas of the cerebral cortex.

The posterior half of the lateral nuclear mass (nuclei ventralis posterior, lateralis posterior and dorsalis) projects to the parietal lobe with a well organized arrangement. Exps. 6 and 8 conclusively show that the postcentral convolution derives its afferent thalamic fibres exclusively from the nucleus ventralis posterior. It is also apparent from these experiments and also from Exp. 7 that the arrangement of the radiation to this gyrus parallels that to the precentral convolution, i.e. the medial portion of the nucleus ventralis posterior (the nucleus ventralis posteromedialis) sends its fibres to the lower portion of the gyrus, the paralamellar zone to the paracentral lobule, and the intermediate part of the nucleus to the intervening cortex.

The medial portion of the nucleus ventralis posterior is frequently spoken of as the arcuate nucleus. Although in places readily distinguished from the remainder of the nucleus ventralis posterior along its lateral and superior margins, it blends imperceptibly with the adjacent lateral portion of the nucleus ventralis posterior. As I have emphasized previously, its significance lies in the fact that it is the thalamic termination of the trigeminal lemniscus first shown experimentally by von Economo (1911). Since the early investigations of

Spitzer (1899) and Wallenburg (1896), the arcuate nucleus has been considered the thalamic termination of the trigeminal pathways. Recently, however, due to difficulties in homologizing the nuclei of the cat with those of the higher primates, it has been suggested that the "pars arcuata" of the ventral nucleus is the site of the termination of the fibres from the brachium conjunctivum (Clark & Boggon, 1933).

The cortical connexions of this arcuate nucleus have not until recently been accurately demonstrated. Sachs (1909) maintained that this part of the thalamus had no cortical connexions. Minkowski (1923–4) found degeneration in it as the result of cortical lesions and concluded that it projected to the central convolutions and supramarginal gyri. Sager (1933) in 1933, suggested that its fibres went to the face sensory area. One year later Walker (1934) found that the sensory cortex from above downwards is represented as a lateromedial arrangement in the nucleus ventralis posterior of the thalamus. He concluded that the nucleus ventralis posteromedialis was concerned in the relay of sensory impulses from the trigeminal nerve. The recent paper by Le Gros Clark (1937) on the connexions of the arcuate nucleus, in general confirm this view.

The nucleus lateralis posterior projects to the parietal lobules since it degenerates after a parietal lobectomy, but not ablation of the postcentral convolution. The projection of this nucleus appears to be intimately related to that of its phylogenetically recent acquisition, the pulvinar. Its projection is probably somewhat scattered, while that of the pulvinar is more intense. In only one experiment (Exp. 4) is the nucleus lateralis dorsalis degenerated, but it is impossible to determine from this case its precise projection. It may project to the inferior portion of the lower parietal lobule as it does in the macaque monkey.

The cortical connexions of the lateral nucleus of the thalamus has occasioned considerable discussion. Most of the previous investigators suggested that it was related to the leg area of the cerebral cortex and projected to both frontal and parietal cortex. Monakow (1895) originally suggested this, and the researches of his pupils, Meier-Müller (1919) and Fukuda (1919), seemed to favour this thesis. Le Gros Clark (1932) pointed out that many theoretical considerations were incompatible with this conception, and it has recently been conclusively shown to be erroneous (Walker 1934; Le Gros Clark & Boggon, 1935).

The pulvinar. The cortical projection of the pulvinar is somewhat widespread and each of the component parts appears to have an independent projection area. Exps. 8 and 9 clearly demonstrate that the inferior nucleus of the pulvinar sends its fibres to the posterior temporal or temporo-occipital region. It seems likely that the upper part of this nucleus projects to the superior portion of this area and the inferior part to the lower portion (Exp. 8). From Exps. 4 and 5 it seems likely that the lateral nucleus of the pulvinar sends its fibres to the superior parietal lobule. The medial nucleus of the

pulvinar probably projects to the supramarginal gyrus, particularly so since in an experiment, not reported here, an isolated lesion in that region gives rise to degeneration in the nucleus pulvinaris medialis.

The projection of the pulvinar has been and still is a rather unsettled question. Early investigators described connexions to the posterior parietal and occipital lobes. A general locus for the projections in that region has come to be accepted. Recent studies have shown, however, that the projection is precise and probably spatially organized. In 1935 Le Gros Clark & Boggon (1935) reported that the inferior nucleus of the pulvinar projected upon areas 18 and 19, but were unable to draw conclusions regarding the remainder of the pulvinar. The following year Walker (1936) concluded that the entire pulvinar projected to the periparastriate areas, but did not give the details of the projection. In 1937 Le Gros Clark & Northfield (1937) presented four experiments to show that the pulvinar projected to the posterior sylvian receptive area of Poliak (1932), except for the inferior nucleus which projected to the temporo-occipital cortex. In his recent monograph on the thalamus of the macaque monkey, Walker (1938*b*) has presented in detail further evidence for his previous statement. He concluded that the inferior nucleus projects to the temporo-occipital region (area 19) and that the nucleus pulvinaris lateralis projects to the posterior part of the superior parietal lobule (area 19 or a transitional area between areas 19 and 5), the nucleus pulvinaris medialis he concluded projects along the anterior margin of the parallel sulcus on either side of the tip of the sylvian fissure (area 19 or the transitional cortex between areas 19 and 7).

IV. SIGNIFICANCE OF THE ARRANGEMENT OF THE THALAMIC PROJECTION

This is not the place to discuss the functional significance of the connexions of each of the thalamic nuclei, but some note concerning the general significance of the cortical projection of this great collecting centre is, I believe, relevant.

The thalamus is composed of at least three functionally distinct nuclear groups, each of which plays a somewhat different role in the integration of sensation. The midline nuclei, relatively constant throughout the mammalian scale, and related not to the cerebral cortex but to other diencephalic structures, are probably concerned with phylogenetically old sensory modalities. A second group of thalamic nuclei are those receiving fibres from afferent thalamic tracts and projecting to the cerebral cortex (the nuclei ventralis posterior, ventralis lateralis, and the geniculate bodies). Phylogenetically the first two of these nuclei develop with the progressive elaboration of the limbs and the latter with the development of the special senses of vision and audition respectively. They are probably related to the relay and elaboration of the elementary sensory impulses. A third group of thalamic nuclei (nuclei lateralis dorsalis, medialis dorsalis and the pulvinar) are those of recent phylogenetic development, a group which does not receive fibres from the main afferent

somatosensory tracts but which projects to the cerebral cortex. They probably have a role in the elaboration of the elementary impulses which enter their adjacent nuclei, before relaying to cortical areas of recent development where further integration into complex concepts takes place.

Through these three groups of nuclei the thalamus receives incoming stimuli, integrates them to varying degrees and then relays them to other subcortical ganglia or to the cerebral cortex. In this way the crude stimuli received by the peripheral end organs become elaborated and are presented to the highest hierarchy of the central nervous system, the cerebral cortex, as complex and at least partially synthesized impulses. However, whereas the elementary sensations do not have isolated representation in the cerebral cortex, the spatial relations of the body are sharply localized. Hence it is possible for an individual finger to be specifically involved by a cortical lesion. It will be apparent now that this spatial relationship is due to the localization of the parts of the body throughout the entire sensory system from the periphery to the cerebral cortex. For example, the sensory fibres from the leg, i.e. pain and temperature through the anterolateral tracts, touch, vibration and position sense through the posterior columns, and other proprioceptive modalities through the spinocerebellar systems—all reach the lateral nuclear mass of the thalamus and terminate along its most lateral part juxtaposed to the reticular nucleus. From here cells project to the leg region of the pre- and postcentral convolutions—thus maintaining throughout the entire system a definite spatial relationship. Undoubtedly the phenomenon of local sign is closely related to this arrangement of the fibres from the bodily segments.

The thalamus is thus the mediator to which all stimuli from the outside world congregate, become modified, integrated and relayed to subcortical centres or are distributed to the several parts of the cerebral cortex. The latter is thus kept informed of the constantly changing environment, so that it may affect adequate adjustments for the well-being of the individual.

SUMMARY

The retrograde cell degeneration in the thalamic nuclei was carefully studied after large and small cortical lesions in the chimpanzee. By this method the thalamic projection to the cortex was shown to be as follows:

1. The large medial nucleus (nucleus medialis dorsalis) sends its fibres exclusively to the granular frontal cortex (prefrontal region).

2. The nucleus ventralis lateralis (anterior half of the lateral nuclear mass) projects to the motor and premotor areas.

3. The nucleus ventralis posterior (ventral portion of the posterior moiety of the lateral nuclear mass) sends its fibres exclusively to the postcentral convolution.

4. The projection from the lateral nuclear mass to the central gyri has a precise arrangement. The medial portions send their fibres to the inferior

pre- and postcentral convolutions, the lateral portions to the superior part of the central gyri, and the intermediate portions project to the intervening cortex of the pre- and postcentral convolutions.

5. The pulvinar projects to the posterior portions of the parietal and temporal lobes. The nucleus pulvinaris lateralis sends its fibres to the posterior part of the superior parietal lobule; the nucleus pulvinaris medialis to the supramarginal gyrus, and the nucleus pulvinaris inferior to the temporo-occipital region.

6. The nucleus lateralis posterior has a rather diffuse connexion with the parietal lobules.

7. The anterior nuclei presumably project to the cingular gyrus.

The operations performed in Exps. 1, 2, 3, 5 and 6 were carried out by Prof. John F. Fulton. I am particularly indebted to him, not only for his surgical assistance in these, but also for his counsel concerning all the experiments. I wish also to thank the various other members of the Laboratory of Physiology for assistance in surgical procedures, Drs. E. H. Botterell, T. C. Ruch, H. Widgerson and H. D. Green.

The neuropathological technique in the preparation of these specimens has been conducted in several laboratories. The technical preparation of the brain in Exp. 3 and part of that in Exps. 1, 5 and 6 was carried out in the Department of Pathology, Yale University School of Medicine under the direction of Prof. H. M. Zimmerman. The brains in Exps. 4, 6, 8 and 9 were technically prepared at the Neuropathology Laboratory of Prof. B. Brouwer at Wilhelmina Gasthuis, Amsterdam. That of Exp. 2 was embedded, sectioned and stained at the Neuropathological laboratory of Prof. Percival Bailey, University of Chicago, Chicago. I wish to express my appreciation for the many privileges and courtesies which have been extended to me by these laboratories. It is also a pleasure to acknowledge the careful, painstaking work of the technicians who have assisted in this arduous task, Miss M. Cook, of New Haven, Miss H. S. Davies of Chicago, and Mr Verheusele of Amsterdam. I would also like to express thanks to Dr Cordier of the University of Brussels for his able assistance in the preparation of several additional specimens.

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ABBREVIATIONS

In the illustrations the numbers in the right-hand lower corner are the numbers of the sections from which the drawings were made. These were drawn using a projector, the details being filled in with microscopic control. In the illustrations containing serial drawings that in the upper right-hand corner is posterior and the lower right-hand corner one is the most anterior. The following abbreviations are used:

AH Ventriculus lateralis, cornu anterior
AV Nucleus anteroventralis
BC Brachium conjunctivum
C Sulcus centralis
CC Corpus callosum
CF Fissure calcarina
CM Nucleus centrum medianum (centre médian)
CMm Corpus mammillare
CS Sulcus centralis
F Fornix
FL Fasciculus longitudinalis medialis
GP Globus pallidus
Ha Nucleus habenulae
I Nucleus pulvinaris inferior
IC Capsula interna
IF Sulcus frontalis inferior
IH Ventriculus lateralis, cornu inferior
IOl Nucleus olivaris inferior
IS Sulcus intraparietalis
L Nucleus limitans nucleus limitans
LD Nucleus lateralis dorsalis
LG Corpus geniculatus laterale
LM Lemniscus medialis
LP Nucleus lateralis posterior
LV Ventriculus cerebri
MD Nucleus medialis dorsalis
MG Corpus geniculatus mediale
MT Sulcus temporalis medius
NC Nucleus caudatus
NG Nucleus gracilis
NH Nucleus hypoglossi
NR Nucleus ruber
NST Nucleus nervi trigemini, tractus spinalis
O Sulcus olfactorius
OD Sulcus occipitalis diagonalis
OF Sulcus orbito-frontalis
OS Sulcus orbitalis
OT Tractus opticus
Pa Nucleus paracentralis
PCI Sulcus postcentralis inferior
PCS Sulcus postcentralis superior
PE Sulcus parieto-occipitalis externa
PI Sulcus parieto-occipitalis interna
PL Nucleus pulvinaris lateralis
PM Nucleus pulvinaris medialis
PRI Sulcus precentralis inferior
PS Sulcus precentralis superior
PT Tractus pyramidalis
Pu Putamen
R Nucleus reticularis
S Corpus subthalamicum
SA Sulcus subcentralis anterior
SC Sulcus callosomarginalis
SF Sulcus frontalis superior
SF' Sulcus frontalis superior (aberrant branch)
SN Substantia nigra
SS Sulcus sylvii
ST Sulcus temporalis superior
T Tectum mesencephali
TM Tractus meynerti
TMT Tractus mammillothalamicus
TS Tractus solitarius
TST Tractus spinalis, nervi trigemini
VA Nucleus ventralis anterior
VL Nucleus ventralis lateralis
VPL Nucleus ventralis posterolateralis
VPM Nucleus ventralis posteromedialis

THE BLOOD SUPPLY OF HEART VALVES IN RELATION TO ENDOCARDITIS

By W. F. HARPER

From the Department of Anatomy, London Hospital Medical College

INTRODUCTION

An accurate knowledge of the structure of human heart valves is important as anatomical peculiarities may suggest an explanation of some of the mechanical components entering into the localization of inflammatory processes in these sites.

The mode of bacterial invasion of heart valves has long been discussed, and one can readily appreciate the importance of this problem since the presence of blood vessels in valves is essential for the theory of embolic endocarditis. Rosenow (1912), reviving the old view of Koster (1878), claimed that bacteria reached the valve by way of capillary blood vessels normally present in them, and he stated that when *S. viridans* was injected intravenously into rabbits, bacterial colonies and haemorrhages appeared beneath intact endocardium. This conception was set aside by others who held that atrioventricular valves contained blood vessels only at their bases, and the semilunar valves none. Bacterial embolism to them, therefore, appeared impossible.

It will be seen that two views exist regarding the production of valvular endocarditis; one, that bacterial emboli enter the valves via blood vessels present in them and become arrested at the so-called "closing edge", the other denying the presence of blood vessels in normal valves claims that the condition is caused by bacteria from the main blood stream deposited on the valve.

In valvular endocarditis it is generally accepted that the affected cusps contain a system of blood vessels, but the details and extent of this system are still undetermined. Unless the presence of blood vessels passing well down into the substance of normal valves can be established, they cannot be seriously considered as a primary contributory factor in the pathogenesis of valvular endocarditis.

Dow & Harper (1932), after an examination of forty human hearts, normal to inspection and from subjects of varying ages without a clinical history of heart disease, found that blood vessels were sometimes present in the atrioventricular valve cusps but did not extend into them for more than 3 mm. from the line of attachment to the heart wall. In all adult hearts the distance was usually much less.

The present author has investigated over 100 additional hearts with similar

results. A further point of anatomical interest in relation to chordae tendineae of the third order may be noted here. At the bases of the cusps these are often muscular in appearance, and it was thought that they might be responsible for the conduction of blood vessels from the ventricular walls to the cusps, for their insertions are frequently very close to the attached margins. Examination of cleared specimens and serial sections of injected material, however, showed that their points of insertion are fibro-elastic and avascular (Fig. 1).

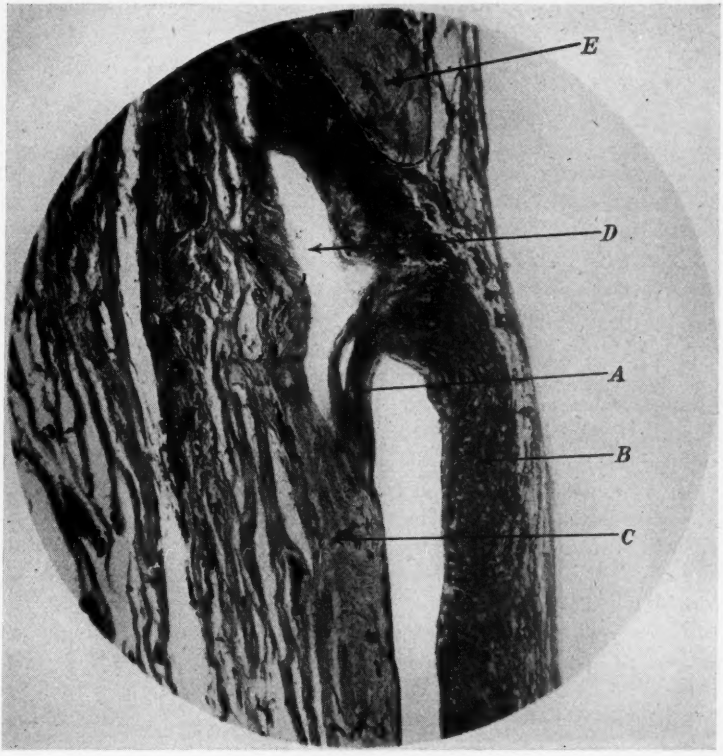

Fig. 1. Longitudinal section through infundibular cusp of tricuspid valve of a male aged 10 years showing a chorda tendinea of the third order. Injected with Berlin Blue, stained with van Giesen. *A*, fibro-elastic substance of chorda (avascular); *B*, fibrosa of cusp; *C*, injected ventricular muscle; *D*, periventricular recess; *E*, injected atrial muscle.

Any vascularity in the cups, therefore, is derived entirely from the atrial side.

The present paper contains observations on the vascularity of the valves of a series of cases of endocarditis, together with the results of certain animal experiments.

MATERIAL AND METHODS

The hearts were kept in normal saline at room temperature until rigor mortis had passed off. After perfusion with warm normal saline they were injected through the coronary arteries, the technique being similar to that employed by Dow & Harper (1932). Rabbit hearts were injected directly through the aorta. The injection masses used were a 3 % aqueous solution of Leichtlosl Berlin Blue 1a, and a 2 % aqueous solution of mandarin pen black, freshly made. The hearts were fixed immediately after injection in a 10 % formalin acetic mixture. Whole specimens of valves were cleared by Regan's modification of the Spalteholz method (1926).

RESULTS

Human

Fourteen cases of ulcerative valvular endocarditis were injected and all exhibited a fairly complete vascularization which extended from the base of the valve cusp to the vegetations, many arterioles of large size being observed. Case 1 is an example of this type.

Case 1. The heart was that of a male, aged 27 years; it was greatly enlarged with considerable dilatation and some hypertrophy of the left ventricle. There was also marked dilatation of the right side. Both cusps of the mitral valve were much thickened and had several large calcareous nodules and broken down vegetations on their atrial aspects. The aortic semilunar cusps were thickened, contracted and rigid. The case appeared to be one in which a chronic endocarditis had followed an acute process. The cleared specimen of the mitral valve (Fig. 2) showed irregular but free anastomoses in the cusps, several arterioles of larger size being present. It will be observed also, that the distal portion of the cusp is quite avascular except where the vegetations encroach on this area. Muscle fibres did not occur in association with these blood vessels.

All three cusps of the aortic valve showed blood vessels extending from their bases to about the middle and visible to the naked eye, while sections provided evidence of obliteration of vessels towards their thickened free edges.

The tricuspid valve and pulmonary semilunar cusps were normal in appearance and contained no blood vessels.

Blood vessels extending from papillary muscles along thickened chordae tendineae were seen in most cases of this type. Vascularization extending to the free margins of aortic semilunar cusps was observed twice (Fig. 3).

Case 2. This specimen which exhibited a tricuspid infection is interesting and rare. The heart muscle was fatty and the heart itself enlarged with much dilatation of the right side. The mitral valve showed chronic endocarditis with thickening and calcification. Its blood-vessel content was very similar to that of Case 1.

Coincident with the mitral lesion a rich vascularity was observed along

practically the whole extent of the thickened free margin of the tricuspid valve (Fig. 4). The vessels supplying it were derived from two sources, viz. by an arteriole from one of the larger vessels in the atrial musculature and by vessels which reached it by passing upwards from a papillary muscle. In addition the septal cusp was supplied at its base by irregular leashes of blood vessels extending downwards for rather less than 5 mm. Muscle fibres did not occur in

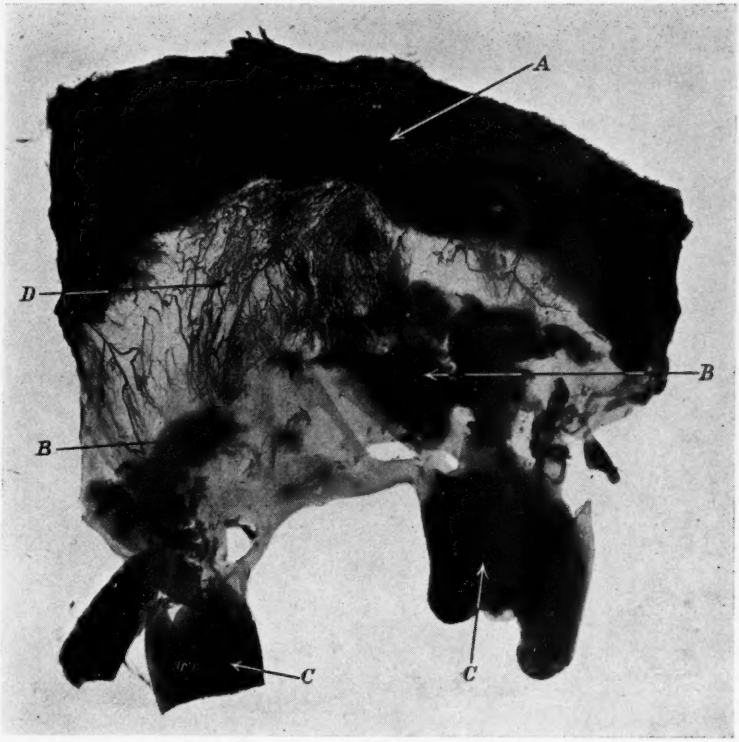

Fig. 2. Endocarditis of anterior cusp of mitral valve in a male aged 27 years. Injected with Berlin Blue and cleared. *A*, atrial muscle; *B*, vegetations; *C*, papillary muscles; *D*, blood vessels in cusp. (After Dow & Harper.)

association with these vessels. The thickened free edge probably represents the actual line of closure in a somewhat shrunken valve.

Case 3. The infection occurred in a female aged 36 years. Post-mortem findings showed moderate hypertrophy of the left ventricle and chronic interstitial nephritis. There was no history of rheumatism or scarlet fever.

The mitral valve exhibited a small nodular elevation, about 3 mm. in diameter, near the junction of the two cusps and a little more than half-way

towards the free edge (Fig. 5). The vascularity took the form of irregular leashes stretching downwards in the direction of the lesion which was itself avascular. Microscopic examination of the leashes revealed many imperfectly filled vessels. No blood vessels were observed in any of the other valve cusps or in chordae tendineae.

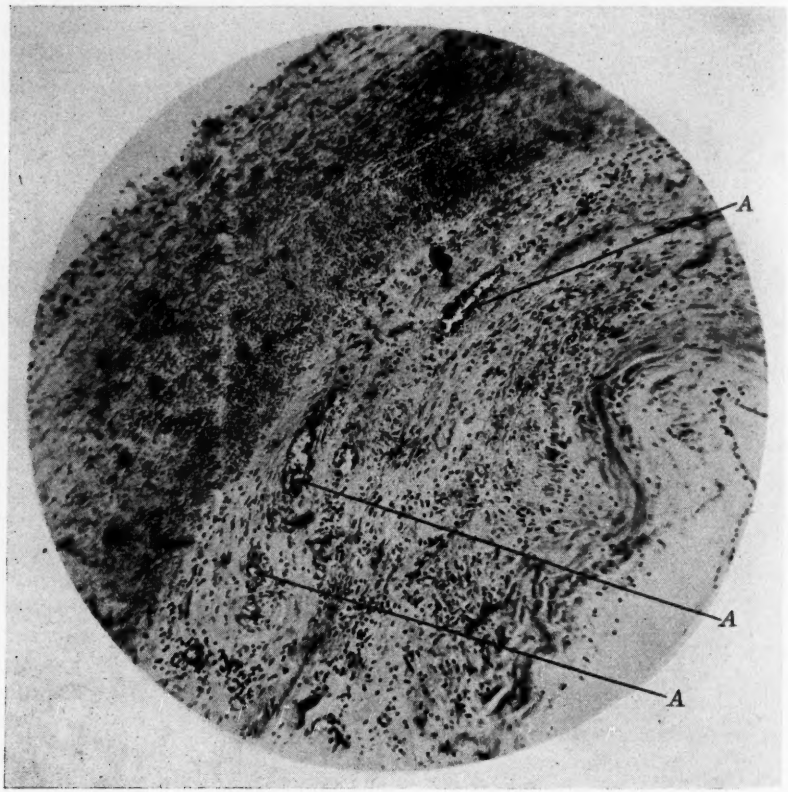

Fig. 3. Endocarditis of an aortic semilunar cusp of a male aged 33 years. Longitudinal section showing blood vessels near free margin. No obvious macroscopic deformity. Coincident with chronic mitral lesion. Stained with haematoxylin and eosin. *A*, new formation of blood vessels in subendothelial layer.

A glance at this vascular pattern gives no idea of time in relation to the disease process, and it was at first thought that the blood vessels demonstrated were "growing towards" the lesion. Examination of serial sections, however, showed that this was not the case, and provided definite evidence of obliteration of vessels near the thickened area. The vascularity, therefore, appears to be in the process of receding or "regressing from" and not growing towards the

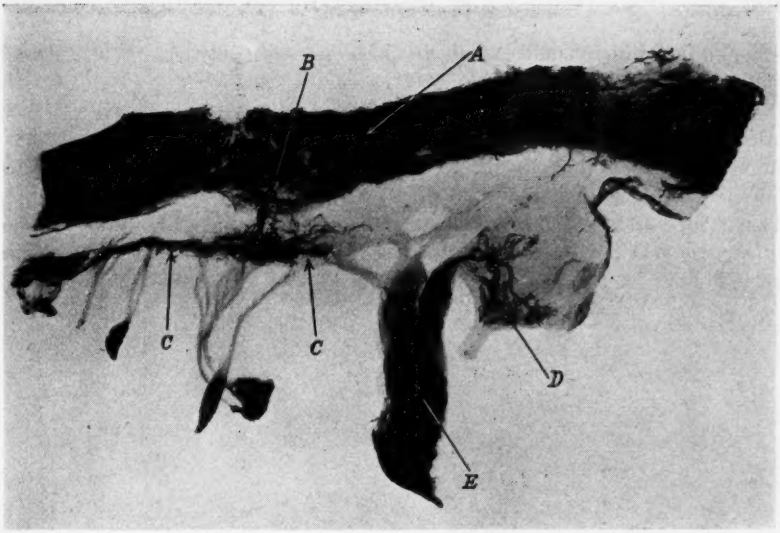

Fig. 4. Endocarditis of tricuspid valve in a female aged 52 years. Injected with Berlin Blue and cleared. *A*, atrial muscle; *B*, arterioles passing to thickened free margin; *C*, *D*, blood vessels from papillary muscle, *E*. (After Dow & Harper.)

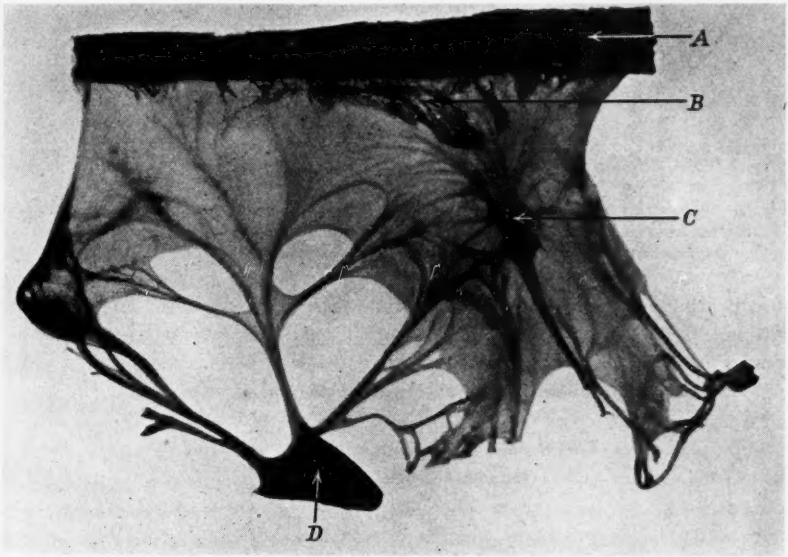

Fig. 5. Endocarditis of mitral valve in a female aged 36 years. Injected with mandarin pen black and cleared. *A*, atrial muscle; *B*, new formation of blood vessels; *C*, nodular thickening; *D*, papillary muscle.

lesion, a fact which would explain the improper filling of the vessels observed at the first examination.

Case 4. This specimen was obtained from a male, aged 39 years, who died of cerebral haemorrhage. The heart showed hypertrophy of the left ventricle, slight dilatation of the right side and chronic interstitial nephritis. In the heart muscle there was moderate fibrosis. The Wasserman reaction was positive. The endocarditis was limited to the mitral valve which showed a nodular thickening near the centre of the anterior cusp and a smaller area of thickening at its junction with the posterior cusp (Fig. 6). Blood vessels extended

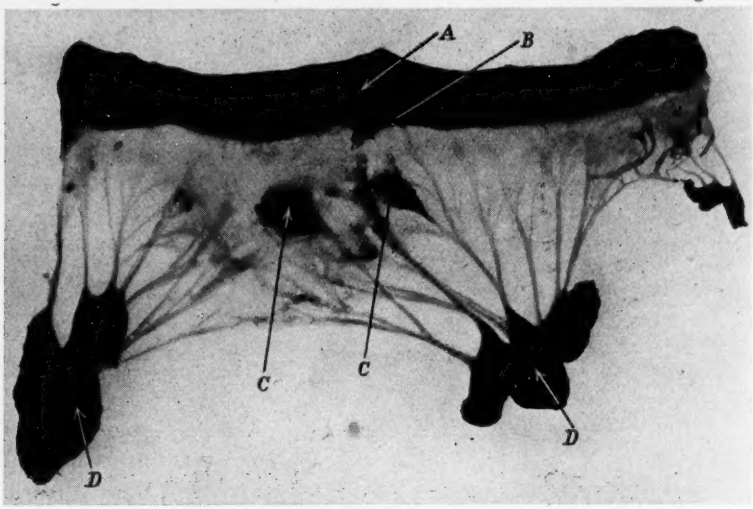

Fig. 6. Endocarditis of mitral valve in a male aged 39 years. Injected with mandarin pen black and cleared. *A*, atrial muscle; *B*, new formation of blood vessels; *C*, nodular thickening; *D*, papillary muscles.

downwards into the valve in the direction of these thickenings. This specimen has not been sectioned, but it is probable that blood vessels have receded from the site of the lesions as in Case 3.

The tricuspid and semilunar valves were normal.

The blood vessel and myocardial content of the heart valves of the rabbit

The atrioventricular valves. No system of blood vessels was found throughout the whole extent of any of the cusps. The vessels usually terminated with the atrial muscle at the bases of the cusps and formed there a terminal capillary network. Tiny vascular extensions associated with prolongations of the atrial muscle into the cups were common. These were relatively longer and more

numerous than the somewhat similar extensions met with in the atrioventricular valves of young human hearts. They varied in length from 1 to 2 mm. and from two to six were present in a cusp (Fig. 7). Microscopic examination of cleared specimens showed terminal capillary loops and atrial muscle in each extension. The blood vessels in these were in most cases derived from twigs which could be traced to the annular branches of the left and right coronary arteries higher up in the atrial muscle. This peculiarity occurred in rabbits

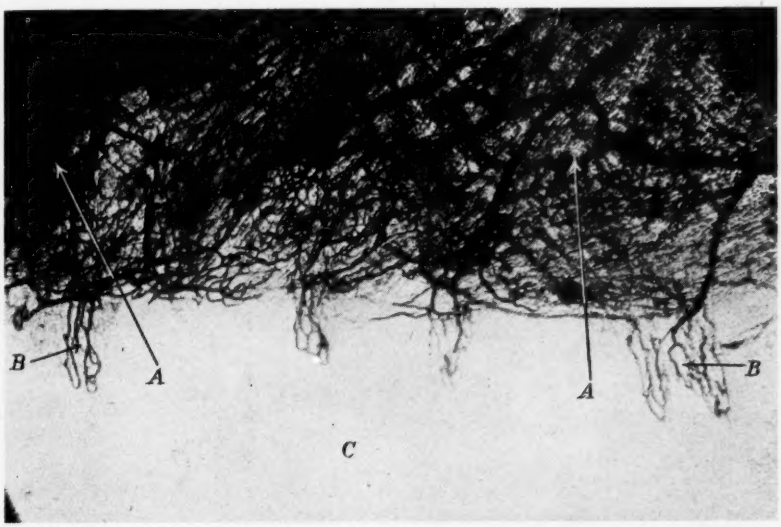

Fig. 7. Anterior cusp of mitral valve of rabbit. Injected with Berlin Blue and cleared. *A*, atrial muscle heavily injected; *B*, vascularized muscle extensions; *C*, avascular fibro-elastic portion of cusp.

of all ages, and as no evidence of muscle regression was observed it would appear that these extensions are of a permanent character.

The aortic and pulmonary semilunar cusps were avascular.

The hearts of over 100 rabbits of different breeds, including the wild rabbit, were investigated.

EXPERIMENTAL

The rabbit has been employed extensively in attempts to produce valvular endocarditis, and many workers have claimed success with intravenous injection of certain bacteria. The lesions described had mainly the features of an acute process and, as far as can be gathered, were associated with blood vessels. Most workers have regarded the process as embolic and have considered the vessels the pathway of infection. They were not concerned with the minute anatomy of the normal cusp.

The results of my investigation indicate that the blood vascular pattern of rabbit valves bears a close resemblance to that obtaining in the human. In both there exists normally only a very scanty supply at their bases. It has also been shown that in human endocarditis the affected cusps become vascularized by new formation of blood vessels down to the site of the lesions. Further, there is definite evidence that a new formation of blood vessels may regress from lesions leaving little or no trace. It seems unlikely, therefore,

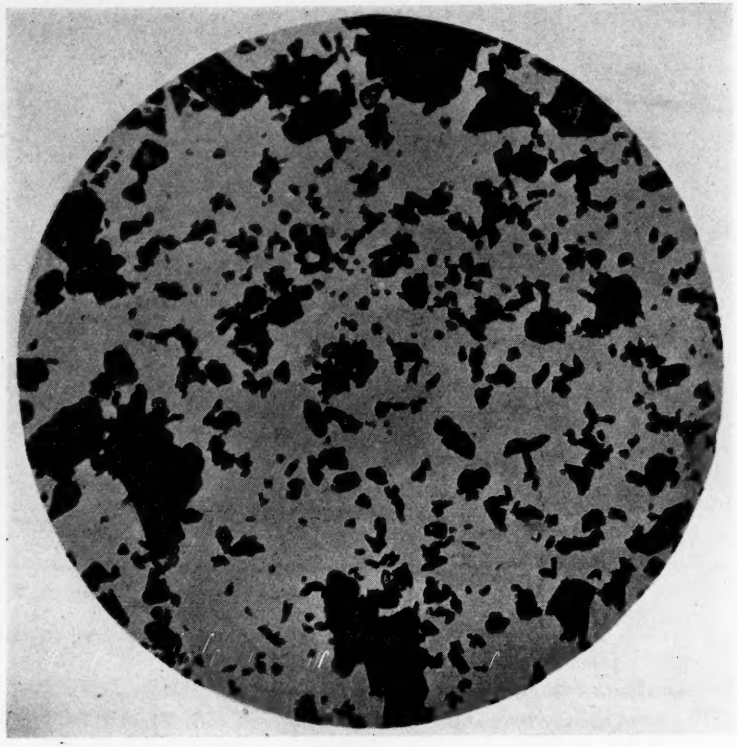

Fig. 8. Photomicrograph of "aleuronate" used for intravenous injection.

that embolic endocarditis is immediately possible either in the human or in the rabbit. It was thought that some light might be thrown on this problem if by experimental means a downgrowth of new vessels could be produced in rabbit valves.

For this work it was considered advisable to use only one breed, and Large Blue (English) rabbits were selected. The investigation of blood vessel and myocardial content of the heart valves of twenty presumably normal animals gave results similar to the above.

"Aleuronate" was used to produce mechanical damage to the valves. It was in the form of a yellowish brown powder almost insoluble in water. Aleurone is found in many seeds in the form of amorphous and pseudo-crystalline granules. Several proteins have been found in aleurone, the most common being globulins, but the composition differs in different plants. The actual plant source of the "aleuronate" used in these experiments was not traced. The pseudo-crystalline fraction differed from true crystals in the

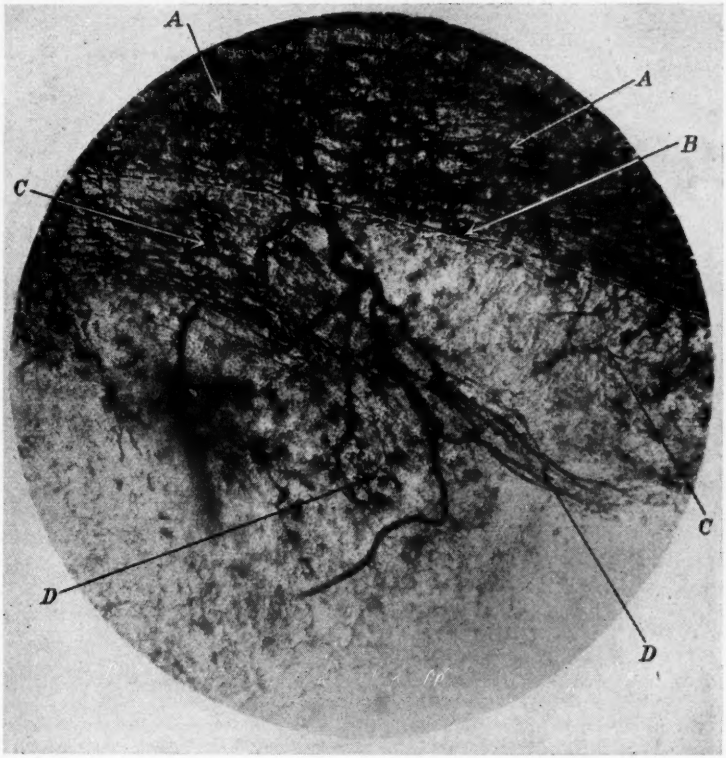

Fig. 9. Infundibular cusp of tricuspid of rabbit, Series 1. Injected with mandarin pen black and cleared. *A*, atrial blood vessels; *B*, line of attachment of cusp; *C*, new formation from atrial vessels, and *D*, from a vascular extension.

inconstancy shown by their angles (Fig. 8). It was insoluble in water but dissolved in a dilute solution of potassium hydroxide and constituted by far the greater part of the powder.

Several tentative injections of different suspensions of "aleuronate" in sterile normal saline were made before a definite series of experiments was set up. The suspension finally employed was made up as follows: 5 g. of

"aleuronate" were added to 25 c.c. of cold sterile normal saline and the mixture stirred well. Of this suspension 5 c.c. were then injected into the marginal ear vein of the animals before sedimentation had begun. Blood cultures were made from each animal before administration of the innoculum and before killing, all with negative results. The animals were killed by means of coal gas.

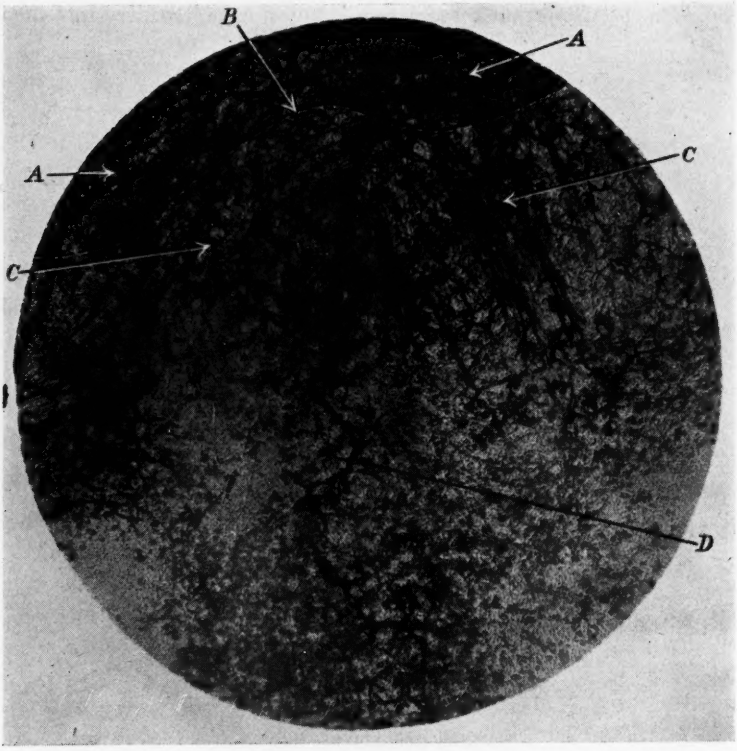

Fig. 10. Septal cusp of tricuspid of rabbit, Series 2. Injection mass and lettering as in Fig. 9.

Effect of intravenous administration of "aleuronate" on the heart valves of the rabbit

Series 1. Five rabbits. Each received an initial injection of 5 c.c. followed after an interval of 3 days by daily injections of 5 c.c. each continued for 7 days. On the 12th day after the first injection all the animals were killed, the hearts isolated and injected. Fig. 9 shows the arrangement of the blood vessels near the base of the infundibular cusp of the tricuspid valve of one of the animals. The atrial vessels at the attached margin of the cusp no longer have the regular formation and tiny new formed vessels can be seen extending

into the cusp. One of the larger arterioles supplying a muscular extension is also less regular in its distribution, several small vessels passing distally from it into the fibro-elastic portion of the cusp.

In all hearts of this series there was similar evidence of a new formation of blood vessels in the fibro-elastic substance of the tricuspid valve. The mitral and semilunar cusps were normal.

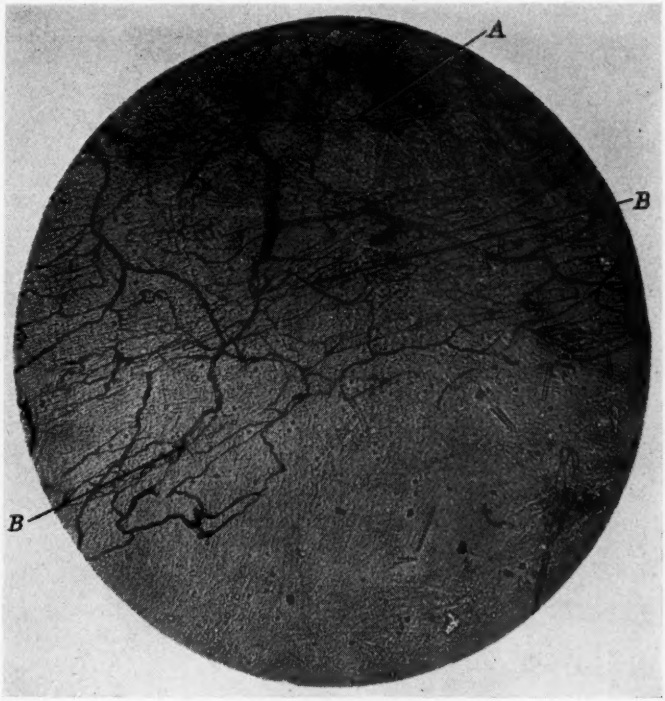

Fig. 11. Tricuspid valve of rabbit near junction of septal and infundibular cusps showing new formation of blood vessels 7 weeks after administration of "aleuronate", Series 3. Injected with mandarin pen black and cleared. *A*, line of attachment of cusp; *B*, new blood vessels.

Series 2. Four rabbits. Each animal received the same number of injections over the same period as in Series 1. Twenty-three days elapsed between the last injection and the time of killing. Fig. 10 shows the arrangement of the few blood vessels in the septal cusp of the tricuspid valve. An increase in number and greater irregularity of newly formed vessels subjacent to the area of contact of the cusp can be observed while freely anastomosing offshoots from the edge of the atrial vessels are also well marked.

The dark mottled appearance over the damaged cusp is due to surface

staining by the injection mass. It is impossible to remove this without further injury to the delicate valve cusps. Improper filling of vessels may be due in part to the slightly lower pressure employed in order to avoid bursting.

No new formation of vessels was observed in the mitral and semilunar valve cusps.

Series 3. Four rabbits. Each animal received an injection of 5 c.c. daily for 10 days. Two were killed at the end of the 7th week and two after the

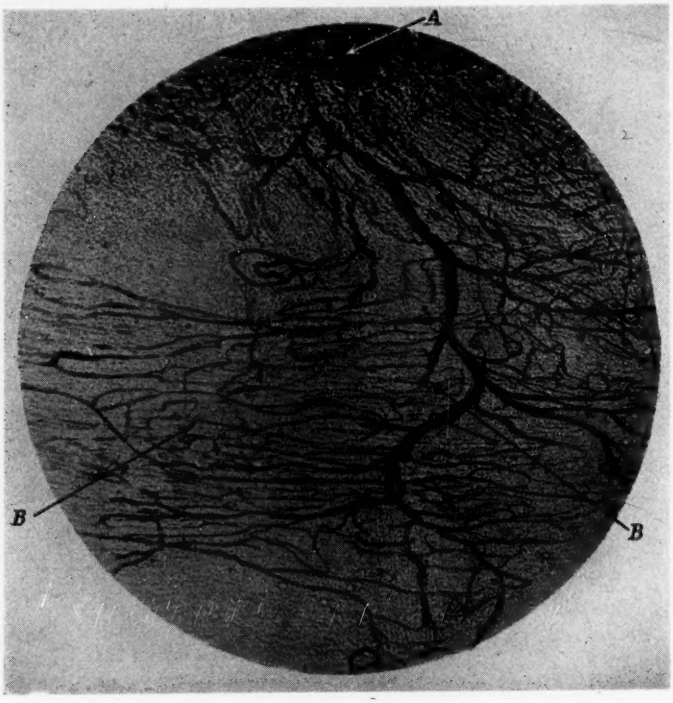

Fig. 12. Tricuspid valve of rabbit showing new blood vessels 12 weeks after administration of "aleuronate". Injection mass and lettering as in Fig. 11.

elapse of 12 weeks. Fig. 11 shows the vascular pattern at the junction of the septal and infundibular cusps after 7 weeks, and (Fig. 12) in the same situation at the end of the 12th week. The new vessels are rather less profuse than in the other series but they are more regular in their arrangement. They extended into the cusps in certain portions for two-thirds of the distance to the free margin. No change was observed macroscopically on the endothelium of these cusps.

The mitral and semilunar valves were normal.

These experiments indicate that a new formation of blood vessels can be produced in the tricuspid valve of the rabbit by the intravenous exhibition of "aleuronate" in suspension, and it is reasonable to conclude that they have been "called out" in response to injury of the valvular endothelium by this particulate matter. It is also indicated that many of them persist for at least 3 months, and by that time the vessels are well established and definite venous channels can be made out.

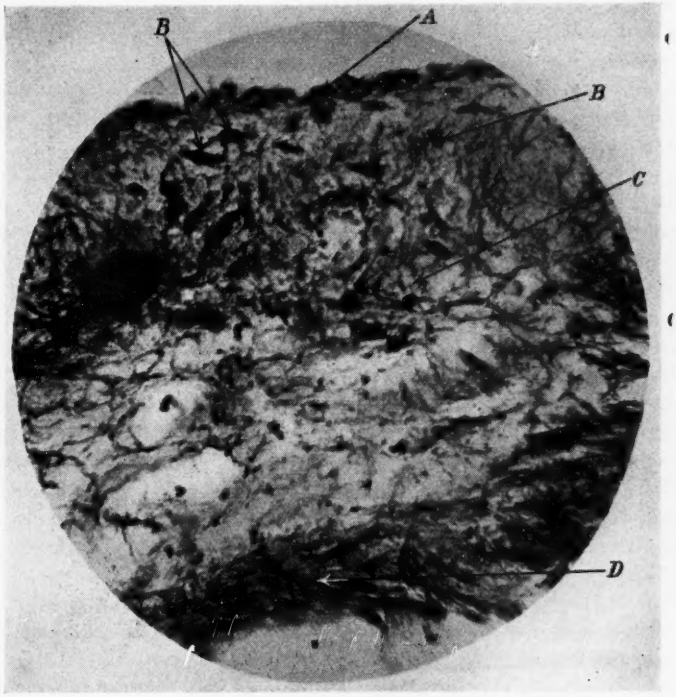

Fig. 13. Longitudinal section through "aleuronate" damaged septal cusp of tricuspid valve of a rabbit, Series 2. *A*, damaged endothelium near base of cusp; *B*, new blood vessels; *C*, fibrosa; *D*, ventricularis.

The normal endothelium of rabbit cusps consists of flattened cells with scanty protoplasm and moderately dense nuclei. In cusps damaged by "aleuronate", irregularity and partial desquamation of this zone occurs (Fig. 13). The cell nuclei increase in size and there is cell proliferation in the subjacent fibrosa. The mononuclear cells in this stratum increase in number and show a scanty protoplasm. This damage readily induces a downgrowth of new blood vessels. But this may also take place without actual shedding

of the endothelium and, under these conditions, the subendothelial layer, before it becomes vascularized, presents in parts a somewhat oedematous appearance. In this connexion it is interesting and perhaps significant, that the primary alteration of endocardium in human endocarditis is due to an inflammatory oedema of the substance of the affected cusp which precedes the deposition of platelets and the formation of vegetations. It may be noted further, that desquamation in "aleuronate" damaged cusps occurs over an area on the valve, this being particularly noticeable on the septal and infundibular cusps. It extends from near their bases as far as the junction of the middle and lower third. Thus there appears to be an actual area and not merely a line of contact when the cusps are in apposition.

Examination of the viscera of the experimental animals showed that much of the innoculum is retained in the pulmonary circulation and that a marked fibrosis occurs in the spleen.

DISCUSSION

Certain results and views with regard to the production of experimental endocarditis, in so far as they concern the present work may be noted here. Shaw (1904) and Poynton & Paine (1900, 1913) claimed to have produced endocarditis in rabbits by the intravenous injection of large doses of streptococci. Rosenow (1928) stated that he produced it in a similar way, and believed that the process was by embolism of blood vessels normally present in rabbit valves. He stated, further, that the site of the lesion is determined by a certain quality of the organisms acquired in the individual from whom they were obtained. He assumed, that is to say, that certain streptococcal strains have a selective action on tissues and described the phenomenon as "the elective localization of streptococci". His evidence as regards experimental endocarditis consisted for the most part of small haemorrhages within the valve substance several days after injection. Evidence in support of Rosenow was advanced by Bargen (1923) and Haden (1923), but Henrici (1916), Moody (1916), Detweiler & Maitland (1918) and Topley & Weir (1921) have stated that the source of the organism has no influence on its pathogenic effects. Detweiler & Robinson (1916) were of the same opinion, and noted further that the deposition of bacteria in the valves seems in some cases to take place on the surface, while in others it appears to be embolic.

During the process of immunization with pneumococci Wadsworth (1919) observed the development of chronic endocarditis in horses, and Mair (1923) described a similar condition in rabbits, and he observed its development in seven out of ten animals which had received a series of injections of autolysed pneumococci followed after 2 months by another series of living pneumococci. Dietrich (1926) also observed that endocarditis was more easily produced in animals which had been treated with injections of the same organism, and thought that this was due to sensitization of the valvular tissues. Wright (1926, 1927) has shown that although it is possible to produce endocarditis

in this manner, it can be done only with great irregularity and he considered that its production may be due to slight injury to the valve rather than to specific sensitization.

The vascularity in the valves of the cases of human endocarditis described in this paper is obviously very different from that obtaining in the normal. The vessels are not associated with muscle, as are the very much shorter extensions in normal valves, but run in the fibro-elastic substance of the cusps. They represent a new formation of vessels. They do not show, however, the orderly arrangement which Gross (1921) stressed as constantly occurring in endocarditis, an arrangement which he stated bore no resemblance to the irregular vascularization of granulation tissue. It is possible that the blood vessels observed by Gross *et al.* (1926, 1928), though distinctly of the arterial and venous types, may nevertheless have arisen as granulation tissue vessels. The results presented in this paper show that blood vessels can grow well down into the substance of a valve.

The most probable explanation of the presence of blood vessels extending into the valves beyond their normal limit is that they are the result of previous injury. The precise nature of the injury in the human is not clearly understood. The resulting downgrowth of blood vessels, while advantageous at the time, undoubtedly lays the valve open to additional infection along these channels. The work of Wadsworth (1919), Mair (1923), Dietrich (1926) and Wright (1926, 1927) is strongly suggestive of injury to the valves leading to the development of endocarditis, and has led the writer to study the effect of immunization on the heart valves of the rabbit. The results, to be incorporated in a future paper, indicate that during the process the structure of the atrio-ventricular valves, including their vascular content, is materially altered.

From anatomical findings it would appear that embolism cannot be immediately responsible for the production of endocarditis, for in both the human and the rabbit there exists only a scanty vascularity at the bases of the valves, unrelated to the sites where vegetations usually develop. In damaged valves with resultant increased vascularity from downgrowth of new blood vessels, embolism could, however, be responsible for the initiation of an endocarditis. It is well known that varying degrees of healing may occur in endocarditis. Cases 3 and 4, recorded in this paper, are examples of this; the new blood vessels found in these cases have fulfilled their function and have become obliterated in the healing process. The extent and persistence of blood vessels in diseased valves will thus depend in part on the state of the lesion.

The mechanical contact of the cusps at their area of apposition may be a factor in the genesis of the condition. Howell (1900) has estimated that the cusps of the atrioventricular valves are compressed just over $10\frac{1}{2}$ hours a day, thus offering a possible opportunity for injury and the arrest of pathogenic organisms.

The question of lymphatics in heart valves has also a direct and important bearing on the problem, since in some way it may play a part in the explanation

of their reaction to pathological states. The subject has engaged the attention of many workers and the results are again somewhat controversial. Aagaard's monograph (1924) is the most comprehensive treatise on the subject. His findings regarding human heart valves were entirely negative although he succeeded in injecting undoubted lymphatic capillaries in other mammalian heart valves. "Dans le cœur humain", he observed, "je n'ai jamais pu injecter, ni chez le nouveau-nés, ni chez les enfants, ni chez les adultes, des vaisseaux lymphatiques dans les valvules ou le long des cordages tendineux, sans égard à l'endroit que je choisissais pour faire la piqûre." These findings are in close agreement with those of certain authors, notably Nussbaum (1912), Darier (1888) and Dow & Harper (1932), regarding the blood vascular content of normal human heart valves, and it may be as Blair (1925) has suggested, that their apparent isolation from these systems bears some relation to the localized nature of the lesions in septic endocarditis.

SUMMARY

1. The blood supply of normal human heart valves is limited to extensions of not more than 3 mm. into the bases of the cusps.

2. In human endocarditis the affected cusps become vascularized by new formation of blood vessels down to the site of the lesions. There is evidence that in certain cases the new-formed vessels regress, leaving little or no trace.

3. It would appear that the primary attack on heart valves is from without, resulting in a downgrowth of new blood vessels into them. In the genesis of endocarditis, therefore, it is more reasonable to presume that a combination of recent injury and coincident blood infection is necessary, rather than to assume a selective activity on the part of bacteria.

4. The heart valves of the rabbit conform to the human type as regards their vascularity.

5. Experimental evidence is put forward to show that a new formation of blood vessels can be induced in the heart valves of the rabbit by mechanical damage.

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THE TENDON OF THE LATERAL PTERYGOID MUSCLE

By J. A. HARPMAN and H. H. WOOLLARD
Anatomy Department, University College, London

The muscles of the jaw must necessarily undergo some modification in the change-over from the Sauropsidan to the mammalian mandibular articulation. In this connexion the peculiar insertion of the lateral pterygoid muscle has attracted attention to the development of its tendon. It had been suggested by Gaupp especially that originally the adductor mandibulae (the medial portion of which most authors regard as the homologue of the lateral pterygoid, vide Edgeworth) was inserted into the articulare. This would suggest that in mammals evidence might be found of this earlier attachment of the lateral pterygoid muscle to the malleus (articulare). In passing to the malleus the tendon of this muscle would have to lie between the condyle of the jaw and the glenoid fossa of the temporal bone. Thus the tendon would be compressed between these two and in this way the origin of the meniscus or disc of the temporo-mandibular joint might be accounted for. Observations in support of these views were put forward especially by Kjellberg and they were adopted by Vinogradoff and others.

The difficulty in accepting these suggestions lay in the fact (which Gaupp himself pointed out) that they were not supported by the examination of embryological material nor by the dissection of primitive mammals. Indeed the investigators already mentioned, as well as Lubosch and others, dwelt on the probability of these relations of the lateral pterygoid tendon, but point out the lack of evidence for their support. Gaupp adds the further observation which cannot be denied, that the exact homologies of the muscles of mastication in the Sauropsida and the mammals are far from certain.

This problem of the peculiarities of the lateral pterygoid has been approached by the examination of selected adult specimens drawn from monotremes, marsupials, primitive and higher eutheria and by the investigation of embryological material from similar types where available. Our own material has consisted of embryological series of rats, guinea pigs, pigs, and also human material. It is the human embryo which is especially interesting for its bearing upon this problem. Gaupp, Kjellberg, and Vinogradoff also investigated embryonic human material. Kjellberg describes how in a 55 mm. human embryo he found evidence of a connexion between the lateral pterygoid muscle and the malleus. This was not seen by other observers. They felt still less disposed to accept the observations of Kjellberg because their other embryonic series which were more

abundant and closer together also failed to reveal any evidence of this connexion with the malleus.

Our own experience is in accord with the statement that non-human embryos do not show any definite trace of a connexion between the tendon of the external pterygoid and the malleus, for we failed to find it in any of those studied by us. The human material, however, does support quite definitely the observation recorded by Kjellberg.

If the tendon of the external pterygoid was formerly inserted into the malleus (articulare) it then must have passed in close proximity to the upgrowing condyle of the dentary as this approaches the squamosal. Since Meckel's cartilage is first somewhat medial and then becomes dorsal and cranial to the condyle, it might be anticipated that the tendon would pass between these two bones, perhaps a little on the medial side of the interval between them. Though the investigators previously mentioned state that the disk is probably derived from the tendon having been nipped within the articulation, nevertheless all of them in recording their actual observations say that the disk arises from the connective tissue between the bones, that the joint cavity is a secondary formation and it is implied the connexion of the disk with the tendon may be secondary. The doubts about the origin of the disk so raised are further reinforced by the observation that sometimes there is no disk at all and some mammals have only a single cavity though of course a bilocular cavity is the general rule. It is true that some of the above writers do occasionally refer to the fact that the muscle fibres of the external pterygoid are connected with intra-articular parts of the disk, but none furnishes evidence that the disk is certainly derived from the nipping of the tendon. We believe, however, that human embryos do show adequate histological evidence of tendon and muscle fibre within the joint as well as the connexion with the malleus as already mentioned. For this purpose the human embryo at a length of 58 mm. offers completely satisfying evidence. Two small points may have helped us in securing the evidence we have mentioned. In addition to the usual sagittal series we also prepared a horizontal series which fortunately gave us a relatively long sweep of the tendon, and further our series was stained with the Van Gieson method, thus giving us specific coloration for muscle, tendon and connective tissue. At the stage mentioned the number of collagen fibres in the primary connective is few and these form a very wide mesh the contents of which do not take up the fuchsin stain. The tendon fibres on the other are well advanced in their differentiation. Kjellberg, whose observations we confirm, did not illustrate his paper with photographs.

In particular our sagittal sections (see Pl. I, fig. 1) passing through the condyle of the mandible show that medially the muscle fibres of the external pterygoid pass above the inner portion of the top of the condyle. They also show the cartilaginous core of the condyle invested by a broad sheet of connective tissue, and above, this connective tissue sheet covering the condyle is surmounted by a mass of yellow muscle fibres in the middle of which is de-

veloping the pink staining tissue of the tendon. On the medial side of the joint the muscle is bounded sharply by a blood vessel. Muscle and developing tendon then occupy the medial half of the joint region approximately while beyond this in the lateral part of the joint a thinner condensation of connective tissue continuous with the foregoing and distinct from that covering the condyle and the squama is present. There is no sharp boundary to the joint region on the lateral aspect.

The horizontal sections are useful because they enable the relationship of the developing tendon to the joint and the connexion with the malleus to be seen in continuity. Reference to Pl. I, fig. 2 shows the condyle sectioned close to its upper extremity. Its cartilaginous core is surrounded by a thick stratum of connective tissue. The fibres of the external pterygoid muscle are attached to this connective tissue sheath in front of the condyle and along its mesial aspect. From the mesial aspect as well as from the posterior surface of the condyle a dense strand of tendinous connective tissue composed of parallel fasciculi with elongated nuclei passes backwards to be inserted into that part of Meckel's cartilage which becomes the head of the malleus. This same tendinous material can be followed far into the belly of the muscle. In Pl. I, fig. 3, the photograph shows a horizontal section at a higher level than the preceding passing through the connective tissue covering the head of the condyle in its posterior part while just in front of this lie the muscle fibres passing into the developing tendon. The developing tendon above the condyle is actually in Pl. I, fig. 4, cut again slightly higher than fig. 3, and emphasizes still further that quite a large mass of muscle fibres do pass well over the condyle and so must lie between the condyle and the squama and therefore do get "nipped" or compressed between the two bones.

The series of photomicrographs illustrate that the tendon of the external pterygoid muscle fibres does pass through the joint area of the temporomandibular region in the human embryo and further that this tendon is prolonged backwards to the head of the malleus. The fibres of the tendon are developing amidst the muscle fibres in part and it is assumed that they are part of the myoblasts which, instead of developing striations, develop collagen and so the distinctive histological and chemical features of tendon. The sections themselves do not advance any fresh information upon the relation of tendon and muscle fibres. They are, however, consistent with the idea of the common origin of muscle fibre and tendon fibre and the linear continuity between them. It is of interest that the best representation of that behaviour of the tendon of the external pterygoid which the study of the Sauropsidan might lead us to expect should be found in the human embryo. Thus is added one more instance of the preservation of primitive characters in man.

The origin of the disk from the tendon of the external pterygoid, at any rate in part, explains perhaps why in contradistinction to other menisci it is vascular and penetrated by many nerves, and of peculiar histological structure, being a not very dense fibrous disk with a few cartilage cells in it.

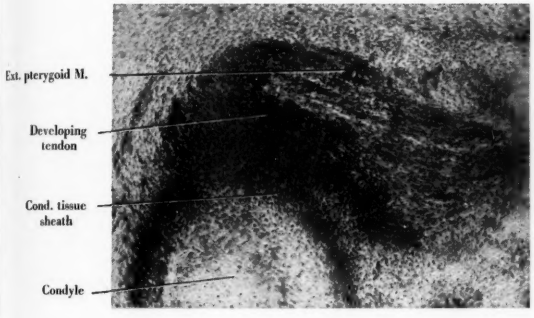

Fig. 1.

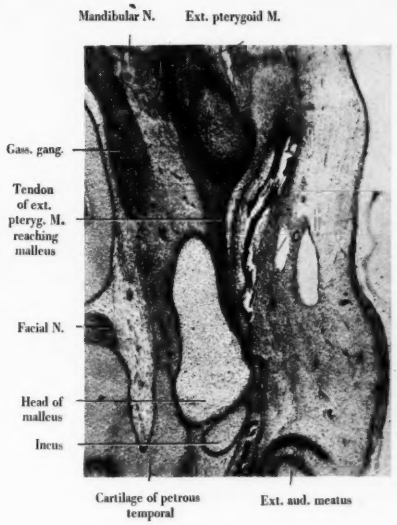

Fig. 2.

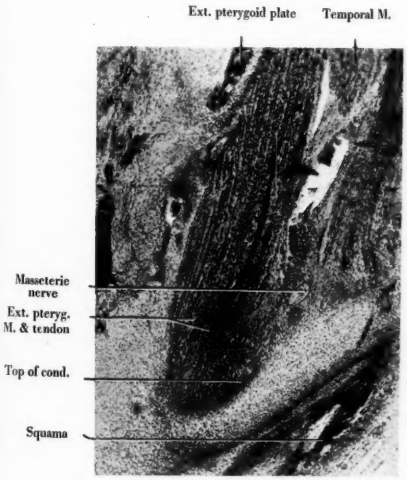

Fig. 3.

Fig. 4.

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SUMMARY

1. The tendon of the external pterygoid, with muscle fibres also in the human embryo passes through the medial part of the temporomandibular joint and is then continued to the malleus.

2. The disk, at any rate in its mesial portion, arises from the tendon of the external pterygoid muscle.

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EXPLANATION OF PLATE I

All figures are derived from a human embryo 58 mm. C.R. length.

Fig. 1. Sagittal section through condyle and squama of the mandibular articulation muscle fibres and tendon fibres are present within the joint. ×140.

Fig. 2. Horizontal section showing insertion of the tendon of external pterygoid muscle into the malleus. ×55.

Figs. 3 and 4 are horizontal sections which show the muscle fibres passing over the condyle of the mandible. ×84.

THE SO-CALLED MAXILLARY ANTRUM OF THE GORILLA

By FREDERIC WOOD JONES

Manchester

The literature concerning the air cavities of the facial skeleton of the gorilla reveals a strange confusion in the nomenclature of the various sinuses connected with the nasal cavity. In brief, there are two large air cavities in the upper jaw of the gorilla and both of these are developed as recesses leading out from the nasal chamber. The confusion in nomenclature of these two cavities dates back to the original description by Owen in 1848.

Describing one of these recesses, he says (p. 412):

> The great maxillary sinus or antrum is chiefly remarkable for its extension upwards, where it swells out the maxillary contribution to the inner wall of the orbit: the nasal aperture of the antrum, of a rounded form and two-thirds of an inch in diameter, is covered by the overhanging inferior turbinal bone: the lachrymal canal terminates at the upper part of the orifice of the antrum, and not in advance of it as in man.

Had this cavity been a true maxillary antrum, it would have differed from that of man, not only by having the lacrimal canal opening into it, but by itself opening below, and not above, the inferior turbinal bone. It is curious that Owen made no comment on this peculiarity. Sonntag (1924) repeats Owen's description, without criticism, and continues to identify the cavity as the antrum of Highmore (p. 131). In another passage, however (p. 396), Owen says:

> The maxillary antrum communicates with the nostrils by a wide aperture, overarched by the inferior turbinal and sometimes also by a smaller opening overarched by the turbinal process above. Its walls being broken away on the left side in a skull of an old male gorilla, shows what appears to be the convex back part of a second wall of the antrum, about half an inch from the outer one.

It is, therefore, evident that in this specimen Owen saw one air space, which opened beneath the inferior turbinal, and bulged into and trespassed on a second, which opened beneath the middle turbinal. Such is the condition typical of the gorilla; it remains to determine the nature of these two outgrowths from the nasal chambers.

Seydel, in 1891, would seem to have been the first to recognize that the large sinus into which the lacrimal foramen opened above, and which in turn opened beneath the inferior turbinate, was in reality the "höhlenartige Ausweitung des Tränennasenganges". In 1902, Keith described and figured the "distension of the nasal canal" in the gorilla and claimed it to be a distension of the inferior meatus, rather than an enlargement of the nasal duct

itself. Keith also showed how this sinus was itself contained within another, larger, sinus which occupied the whole of the posterior part of the upper jaw. Underwood, in 1910, described and figured the "enormous inflation of the nasal duct" of the gorilla which, he says, "looks like an anterior division of the sinus at first sight". In 1917, Bolk, without reference to the work of the previous writers, redescribed the dilated nasal duct of the gorilla and, somewhat unfortunately, named it "Bulla maxillaris". He pictures this bulla as being the equivalent of an anterior ethmoidal cell in *Homo*. Finally, in 1936, Wegner described and figured, in great detail, the whole air sinus system of the gorilla and named the sinus under discussion "Bulla ossea nasolacrimalis".

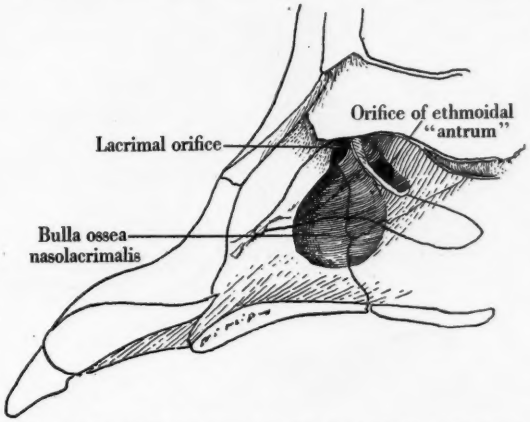

Fig. 1. Median section of the skull of a young gorilla in which the milk dentition is not fully erupted. The suture lines between the maxilla and the ethmoid and palate are clearly marked. The site of the interior turbinate is indicated in outline. The bulla ossea nasolacrimalis and the "antrum" are already developed.

Having arrived at agreement concerning the nature of this sinus, it remains to enquire if the large sinus that opens beneath the middle turbinate of the gorilla is a true maxillary sinus. It is quite definite that, in the orang and in man, the sinus that inflates the maxilla is a true outgrowth from the maxillary portion of the wall of the nasal chamber: and it seems universally to have been assumed by more recent writers, that the large posterior sinus in the gorilla is of the same nature. The question can only be settled by examining the condition in the young skull. In the skull of a young gorilla in which the only teeth fully erupted are the milk incisors and the first milk molar and in which the milk canine and second molar are in process of eruption, the development of the two sets of sinuses is well advanced. The outgrowth of the bulla ossea nasolacrimalis involves the maxilla and the ethmoid (as noted by Wegner (1936)) but it also trespasses, below the ethmoid, on to the palate bone. It, therefore, involves the whole of the posterior part of the maxilla and encroaches behind upon the ethmoid and palate. This creates the condition

that was noticed by Wegner and which was described thus: "Owing to the expansion of the bulla, the aperture of the sinus maxillaris above it seems to be pushed rather high up." Such is a perfectly correct description. The orifice is situated higher up and further back than would be expected if the sinus were in reality a sinus maxillaris. In the adult animal, in which the suture lines are no longer conspicuous, no other diagnosis can be made. In the skull of

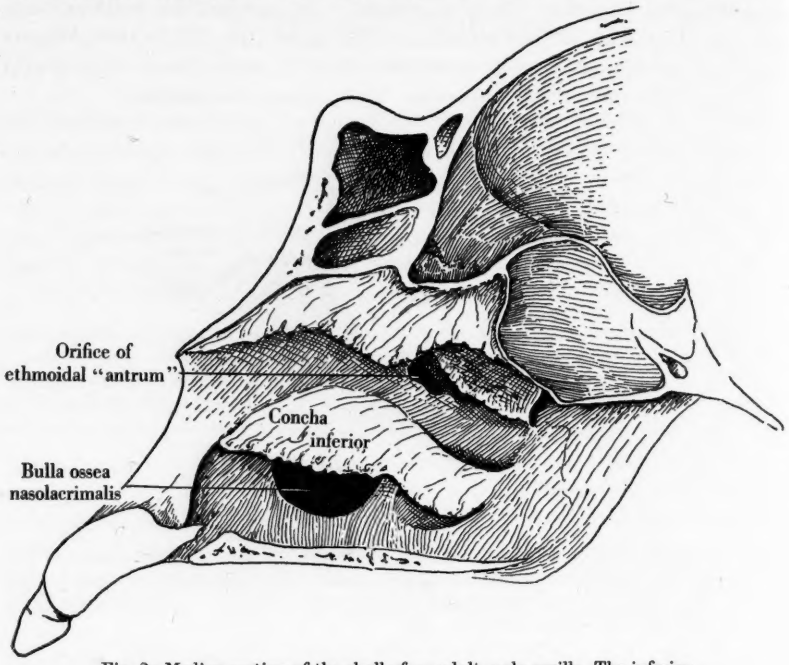

Fig. 2. Median section of the skull of an adult male gorilla. The inferior turbinate is *in situ*.

the young specimen, however, it is at once apparent that the outgrowth of the sinus is through the ethmoid, a long way posterior to the hind end of the maxillary element in the lateral nasal wall. The position of the outgrowth might be described as corresponding to the middle ethmoidal air cell in *Homo*.

As a matter of fact, there is, in the gorilla, no sinus maxillaris, for the site of a maxillary sinus has been usurped by the bulla ossea nasolacrimalis. The sinus which has been described as the sinus maxillaris is in reality a vastly enlarged ethmoidal air cell which has taken the place of the maxillary sinus of the orang and man.

In the young gorilla, in which the milk dentition is not fully erupted, the bulla ossea nasolacrimalis somewhat exceeds in size the ethmoidal sinus. With the further eruption of the teeth, the bulla ossea nasolacrimalis enlarges and the canalis infra-orbitalis, which was situated at first in its roof, comes

(in old male gorillas) to lie free in its cavity as a complete bony tube, in exactly the same way as it does in the true maxillary antrum of the orang. In the young gorilla, a vertical wall separates the bulla ossea nasolacrimalis in front from the rather smaller cavity of the ethmoidal sinus behind; but, with the growth of the jaws, the ethmoidal sinus grows forwards around the hinder and lateral wall of the bulla which thus comes to be contained within the sinus. The growth processes have been so well described and illustrated by Wegner, and the adult condition so well figured by Keith, that further reference to the

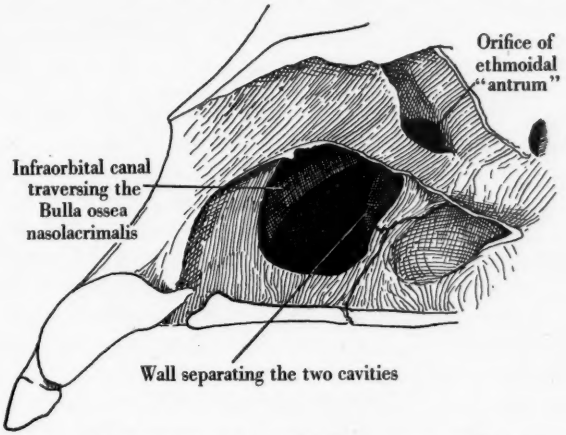
Orifice of ethmoidal "antrum"
Infraorbital canal traversing the Bulla ossea nasolacrimalis
Wall separating the two cavities

Fig. 3. Median section of the same skull as shown in Fig. 2, with the inferior turbinate removed.

anatomical condition is unnecessary. Although Keith described the bulla ossea nasolacrimalis as being characteristic of both gorilla and chimpanzee, I am in agreement with Underwood and Wegner, that in the chimpanzee the condition is somewhat different; for only the inferior meatus is enlarged at the site of opening of the nasolacrimal canal (which resembles that of the macaques in being largely formed by a tubular lacrimal bone).

One must admit with Aubert (1929), that the bulla ossea nasolacrimalis "constitue, chez le gorilla, un caractère de classification très important". But it is equally important to recognize that the absence of a true maxillary antrum and the presence of a greatly enlarged ethmoidal air cell, which occupies the position usually taken by the maxillary antrum, is a still greater distinction of the gorilla.

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THE PRODUCTION OF CORTICAL LESIONS BY DEVASCULARIZATION OF CORTICAL AREAS

By SYDNEY SUNDERLAND

Department of Human Anatomy, Oxford

LITERATURE

The results of Duret's work on the distribution of the cerebral arteries, as determined by injection, gave a cortical as distinct from a basal or deep distribution, with no anastomosis between the two systems. The supply to the basal ganglia was localized by him to those structures, whilst cortical vessels from the pia supplied the cortex by short vessels, and also the cortex and medulla by long perforating channels. The latter passed through to supply not only the immediate subcortical white matter but also the centrum ovale as far down as, but not including, the basal ganglia. Each vessel, cortical or basal, was believed to supply a special territory in an arborizing manner, and it was held that no anastomoses existed between each system of arborizations. These conclusions of Duret (1874) confirmed the existence of end arteries in the brain, as originally postulated by Cohnheim who had previously studied the arrangement and termination of the cerebral arteries by means of an injection technique. Heubner (1874) and Beevor (1908), from injection studies, arrived at a similar conclusion at a later date. It is to be noted that Duret made no mention of any vessels coming from deeper systems to supply the deeper layers of the cortex. It was this work of Duret, the first to approach the subject as a special study, which dominated the teaching of his time and which has persisted and passed down to most of the current text-books of anatomy.

The result of more recent investigators such as Pfeifer (1930), Cobb (1929, 1931, 1932), Craigie (1929–30) and others, whilst confirming the general ground plan of the intracerebral vascular pattern as demonstrated by Duret, are, nevertheless, responsible for certain fundamental modifications of his original conclusions. It is now generally accepted that there does exist a very complex intracerebral capillary and arteriolar anastomosis between all systems, cortical, medullary and basal; so that there are no end arteries in the anatomical sense, although it is well known that such are present in the physiological sense. Although direct anatomical observation reveals the presence of diffuse anastomotic networks, it gives no idea of the efficiency of the collateral circulation present. The effective blood supply to a part and the efficiency of a collateral circulation can be determined only by experiment. It is interesting to note that there is one animal in which true end arteries apparently exist, for Wislocki

& Campbell (1937), describing an unusual manner of vascularization in the brain of the opossum, *Didelphys virginiana*, showed that the brain is supplied by distinct and separate vascular units which do not anastomose with one another.

Opinion still differs on the ultimate destination and distribution of vessels arising from the pia. Pfeifer (1930) believes that there is no clear distinction between short perforating cortical vessels localized to the grey matter, and long perforating cortico-medullary vessels which supply all regions through which they pass before terminating in the medulla. Others, such as Freedom (1935) and Cobb (1932), are convinced that separate cortical and cortico-medullary arteries exist, and the arrangement seen in the specimens used in the present investigation confirm this view. The work of Pfeifer (1930), Cobb (1932) and Freedom (1935) also emphasizes the presence of deep medullary vessels which pass out to reach the deepest layers of the cortex where they anastomose with the capillaries of the superficial system. The origin of such medullary vessels is not given, but apparently they are presumed to come originally from the perforating basal arteries going to the basal ganglia. The significance of these vessels is doubtful. They are said, by those who describe them, to nourish the deeper layers of the cortex. No attempt has been made to prove this experimentally, although Tsang (1936), in a paper on vascular changes following experimental lesions in the cortex of the rat, makes a brief and unsatisfactory reference to this point. He writes, "since the neopallium derives its blood supply mainly from the large trunks on the outer surface of the brain, a perfect ring will cut off the chief vascular channels from the encircled mass. The only source of circulation, apart from the possibility of new blood vessels growing into the region, is through capillary anastomoses with other parts underneath the lesion. This should be a good test of the possibility of forming adequate vascular channels through a limited source of supply. A horseshoe-shaped lesion is also good for the same reason: an example was Rat I. The enclosed cortical mass in both animals was profusely supplied with capillaries." No mention is made of the fate of the encircled mass, although it is stated that it is profusely supplied with capillaries. However, no such condition has ever been observed in the experiments recorded below. It is also impossible to agree with Tsang when he writes that "a horseshoe-shaped lesion is also good for the same reason", for, clearly, no lesion is likely to be adequate for demonstrating anastomoses with deep medullary vessels unless it accounts for the complete obliteration of the superficial vascular supply.

The anatomical and histological features of the vascular supply to the cerebral cortex have certainly been investigated in considerable detail, and reliable information on the course and distribution of the vessels is available. The practical value of such vascular distribution, however, has received little attention. This paper records an experimental investigation of the blood supply of the cortex by obliterating the superficial supply from the pial vessels and

examining the resultant degenerative changes, and also by isolating cortical areas, without interruption of the pial vessels, to determine the part played by deep medullary arteries in the supply of the deepest cell laminae. The objects of the enquiry were to ascertain:

1. Whether the cortex is supplied entirely from the surface, or whether the deeper layers receive a separate or additional supply by deep ascending branches from the medulla.

2. The extent to which it is possible for an effective collateral circulation to be established to any cortical area after interference with its superficial supply.

3. The extent of the effective blood supply of the medullary white matter from the surface of any particular cortical area.

4. The feasibility of producing local cortical lesions by interruption of the vascular supply as opposed to the actual removal of brain tissue.

THE EXPERIMENTAL INVESTIGATION

Macaque monkeys were used. Under nembutal anaesthesia cortical areas were deprived of their blood supply by cutting off the vessels passing to them from the pia with a diathermy. In the isolation experiments, cortical areas were isolated by subpial undercutting.

The experiments

Monkey	Operation	Areas involved	Age of lesion (in days)
O.M. 171	Devascularization	Occipital	5
O.M. 209	,,	Motor Sensory Parietal	7
O.M. 163	,,	Sensory	10
42 A	,,	Frontal	16
O.M. 251	,,	Motor Sensory Parietal Peristriate	83
O.M. 226	Isolation	Motor Sensory Premotor Visual	118
O.M. 164	,,	Visual	46
O.M. 149	,,	Visual Sensory	14

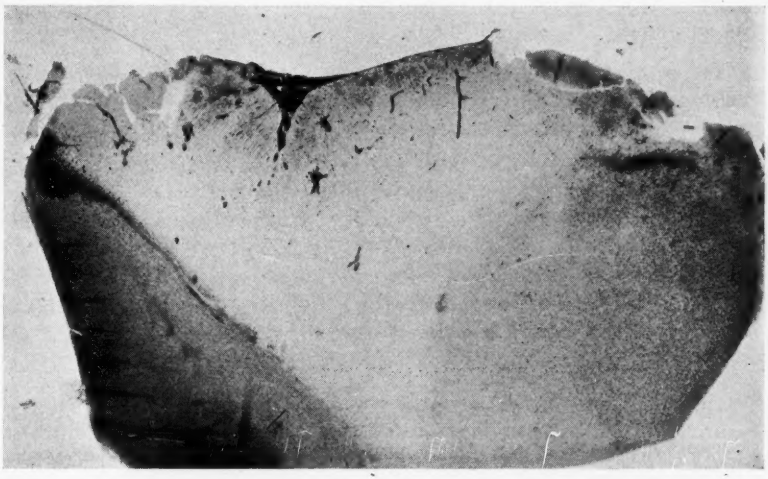
THE HISTOLOGICAL EXAMINATION

The brains were removed and placed in formalin for 24 hours when blocks containing the areas were cut. To examine the changes in the cell structure, sections were cut transversely through the lesion at 15 μ after the blocks had been fixed and embedded. These sections were stained with 1 in 5 Borrel's Methylene Blue in the oven at 58° C., differentiated in Gothard's solution, and mounted in neutral balsam. The state of the capillary bed was investigated by the nitroprusside-benzidene method as elaborated by Pickworth (1934).

RESULTS

In the animals O.M. 171, 209 and 251, the devascularized zones were small, 10–15 mm. in diameter, and unfissured and, since the changes were the same irrespective of the area involved, a general account suffices for all the areas.

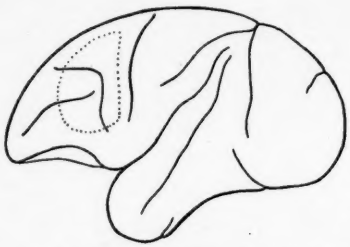
In the recent cases the cortex was completely disintegrated with either complete separation of the degenerated mass, as seen in Pl. I, fig. 2, or with the appearance of a line of separation along which cell reaction was very marked, as seen in Text-fig. 1. The subjacent white matter was disorganized and converted into a pale diffuse staining mass with no marked cellular reaction except at the periphery where a line of separation had appeared (Text-fig. 1). The



Text-fig. 1. Microphotograph showing a lesion in the motor area of approximately the same dimensions as that seen in Pl. I. The encircled area has degenerated but not separated, although the line of reaction along which separation will occur has appeared. The medullary lesion is characteristically wedge-shaped. The section is stained with Borrel's methylene blue. ×10.

extent to which the white matter was involved varied and this appeared to depend in some measure on the disposition of the white matter beneath the cortex. Where a cortical area is accommodated on a narrow and finely folded convolution, e.g. visual area (Pl. I), the medullary core of the convolution is almost completely surrounded by cortex, and all parts of it are in reach of perforating vessels from more than one side. Here most of the subjacent white matter is preserved presumably because of the proximity of supply by perforating vessels from neighbouring cortical areas. A collateral circulation, however, sufficient for the needs of the remainder of the medulla and the cortex superficial to it, was not established, with consequent atrophy of those

parts. When a cortical surface of the same dimensions is involved on the summit of a broader convolution with a deeper substratum of white matter, as in the motor area (Text-fig. 1), the white matter is disintegrated to a depth well below the cortex. Here a stage is reached where an area of white matter becomes so far removed from all but one cortical vascular field, and is consequently so poorly supplied by deep medullary arteries, that when the vascular supply to that cortical field is obliterated, the subjacent white matter suffers the same fate as the overlying cortex. There was a marked vascular reaction at the cortical edges in this case, but in the medullary base this was less marked in accordance with the poorer vascular supply. Many thrombosed vessels were present in the involved area and capillaries were scattered throughout it. These capillaries are not intact but are merely vascular "ghosts" which, because of their structure, have maintained their architecture longer than the softer surrounding tissues which have degenerated into a structureless mass.



Text-fig. 2. A diagram to illustrate the relationship of the lesion in monkey 42A to the sulci of the frontal lobe.

In monkey 42A, the area involved was large, irregularly oval in shape and measuring about 2·5 cm. in its greatest diameter, whilst the line of obliteration crossed two deep sulci in several places (Text-fig. 2). Posteriorly, the encircled cortex showed marked necrosis, extending deep into the white matter almost as far down as the basal ganglia. The medullary vessels were congested with a reactionary dilatation. Anteriorly in the area, however, vessels had entered along the depths of sulci where they escaped damage when the superficial ring was diathermized; as a result, the neighbouring cortex was preserved.

Reparative changes were in evidence in the late lesions. The cortex had completely disintegrated. The base of the lesion was occupied by a still very vascular granulation tissue. The cell infiltration and vascular reaction were denser in the superficial areas than in the deeper. New vessels had entered from neighbouring cortical areas as well as from the subjacent medulla. It is difficult to assess the original medullary damage because of the collapse of the wound edges and the contraction that has approximated the intact cortical edges.

In the isolated cortical areas, apart from a selective atrophy of cortical cells in certain laminae due to interruption of their neurones, the cortex and

the included white matter were normal. There was no granulation tissue present and the blood vessels were normal. In these experiments only the white substance immediately subjacent to the cortex was included. The absence of changes in this isolated medullary tissue indicates that its effective blood supply comes from perforating vessels passing in from the pia.

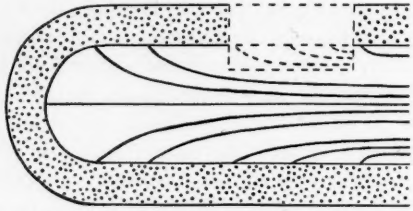
DISCUSSION

The experimental evidence demonstrates that the effective circulation to the cortex is derived entirely from the superficial pial vessels. Any supply from ascending medullary vessels entering the deeper layers of the cortex is functionally insignificant, because, after interruption of pial vessels, the deeper layers of the cortex do not persist, and also because, after complete isolation of the cortex, the cortical changes are those due to an interruption of efferent pathways, and not to any interference with the supply from medullary vessels.

After interference with the superficial supply to the cortex no effective collateral circulation is established either from adjacent cortical vascular plexuses or from deeper medullary systems. The line of demarcation in the cortex is clear cut, and the cortical degeneration corresponds accurately to the encircled area and is not inside it. This would suggest that there is no effective collateral circulation even to immediately adjacent areas. As in experiment 42 A, even the preservation of an intact cortical field inside the obliterated ring could not provide a collateral circulation sufficient to nourish the encircled cortex. Apparently the circulation to any particular area functions as if it were a closed system, so that it may be said that the functional condition is identical with that which has been expressed anatomically in the brain of the opossum.

The relationship of the cortical vessels to the subjacent medulla also receives some explanation from this investigation. It is well known that the superficial cortical vessels supply the subjacent medulla, but this investigation has shown that the functional value of such a supply varies. In narrow, well-folded convolutions the damage following their obliteration is a minimum, because of the effectiveness of the collateral circulation. In less well-folded areas, where the lesion involves the summit of the convolution, the degenerative changes are more extensive so that relatively more of the subjacent white matter is destroyed. If Text-fig. 1, illustrating degeneration in the motor area, is examined, it will be seen that actually the deep region of the degenerated white matter is no further away from the cortex on either side than it is from the involved cortical field. That such a deep wedge-shaped territory receives its supply from a more distant field suggests that the superficial supply is predominantly, though not necessarily exclusively, to the fibres of the cortical area destroyed. It is clear, from the presence of the thrombosed vessels, that the medullary changes are a real manifestation of an interruption of the blood supply from superficial vessels, and that they are not due merely to the

degeneration of the fibres corresponding to the cortical areas involved. It would appear that the perforating cortical vessels follow the relevant fibre pathways into the white substance and accompany them for some little distance, supplying the white substance through which they are passing, before that supply is taken over by other vessels. Whether any other fibres of passage are likely to be present in this medullary zone so supplied is doubtful. The possibility of interference with fibres of passage is difficult to prove; this might be established by investigating for degenerative changes in cortical fields and pathways outside the encircled area which would indicate damage to fibres passing through the involved medulla. Such an examination has not been included in this investigation. The evidence at hand, however, suggests that



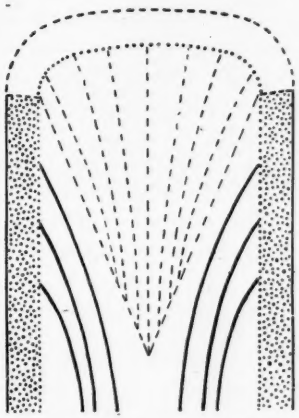
Text-fig. 3. A diagram to illustrate the manner in which the medullary destruction involves only those fibres arising in the devascularized cortex where the lesion is situated on the side of a narrow, well-folded convolution.

the amount of destruction depends both on the site and size of the lesion. For instance, small lesions on the side of a narrow, well-folded convolution, as in the visual area, show little subjacent medullary damage, and it is most likely that this is restricted to that portion of the medulla occupied only by those fibres arising in the involved cortex (see Text-fig. 3). Thus in these cases there is less likelihood of interrupting the fibres of passage from the tip and more distal parts of the convolution, because these fibres will be centrally situated in the medullary core and so will be placed deep to the medulla which degenerates with the cortex. However, in the case of lesions on the summit of broader convolutions it has been seen that the medullary changes occur to a much greater depth, even as far down as the basal ganglia. While it is clear that the cortical vessels can only supply the fibres connected with the corresponding cortical areas for a limited part of their course, the results of the devascularization experiments do suggest that they extend into the medulla as far as the latter is composed *predominantly* of these fibres (Text-fig. 4). From the experiments on the isolated cortex it appears that the supply to the subcortical white matter from adjacent medullary vessels is functionally insignificant.

A comparison can now be made between the methods of vascular interference and cortical ablation in the production of localized cortical lesions.

If cortical lesions are produced by cutting away the cortex with a knife

or diathermy, the depth to which the tissue is removed can be judged only approximately, and it is extremely difficult to repeat a lesion of the same depth, again or in another area. Moreover, if the depth is too shallow, deeper cell laminae will be left intact, whereas if the section is taken too deep fibres of passage are likely to be involved in the lesion, and the results will be consequently affected and difficult to assess in terms of the cortical area actually removed. The associated blood vessels are also definitely interfered with in cortical ablations and this means degeneration of a varying amount of the medulla. Thus cortical ablations localized to the cortex must necessarily produce changes in the medulla at least as extensive and damaging as obliteration of the blood supply. The question under review is whether vascular interference provides a better method for producing lesions localized to the cortex. In this method it is certain that the cortex is completely destroyed. The amount



Text-fig. 4. A diagram to explain the characteristically wedge-shaped formation and appearance of the medullary lesion when the devascularized cortex is situated on the summit of a broader convolution.

of white matter involved depends on the area under investigation. In the case of narrow, well-folded gyri, only the medulla containing fibres from the involved cortex is damaged so that here the method is an ideal one. In other regions, for example the motor, where the medullary involvement is more extensive, although the degeneration involves a medullary area which consists, *at least predominantly*, of fibres arising in the involved cortex yet it is possible that fibres of passage may be destroyed as well. But this would also occur in cortical ablations. In so far as smooth areas of the cortex are concerned, devascularization is preferable to cortical ablation because it is simpler, just as effective if not more so, does not involve instrumental interference with the cortex, and allows an identical lesion to be repeated.

SUMMARY

An investigation into the blood supply of the cerebral cortex and the production of cortical lesions by interference with that supply is recorded. The results indicate that:

1. The cerebral cortex receives its entire effective supply from superficial pial vessels. Ascending vessels from the subjacent medulla such as have been described by some authorities, if they exist at all, are functionally insignificant.

2. When the blood supply to any cortical area is interrupted by obliterating the superficial pial vessels, no effective collateral circulation is established to the area either from deep ascending medullary vessels or from neighbouring cortical areas. Thus, although anastomoses are present, the individual pial vessels function as end arteries.

3. The extent to which the subjacent medulla is supplied from superficial cortical vessels varies. In small lesions involving narrow, well-folded convolutions the medullary damage is a minimum, and almost certainly involves only the fibres arising in the devascularized area. In larger lesions involving the summit of larger, less convoluted areas, the medullary damage extends more deeply. In such cases, although the line of degeneration suggests that the changes involve the territory conveying only fibres arising in the devascularized area, involvement of fibres of passage cannot be definitely excluded.

4. The applicability of this method of producing local cortical lesions is discussed and compared with that of cortical ablation.

ACKNOWLEDGEMENTS

The operations in these experiments were carried out by Prof. W. E. Le Gros Clark. I am extremely grateful to him not only for suggesting the subject of this research but also for his constant advice and kindly asistance throughout the investigations. The microphotographs are the work of Mr W. Chesterman of this department.

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Fig. 1.

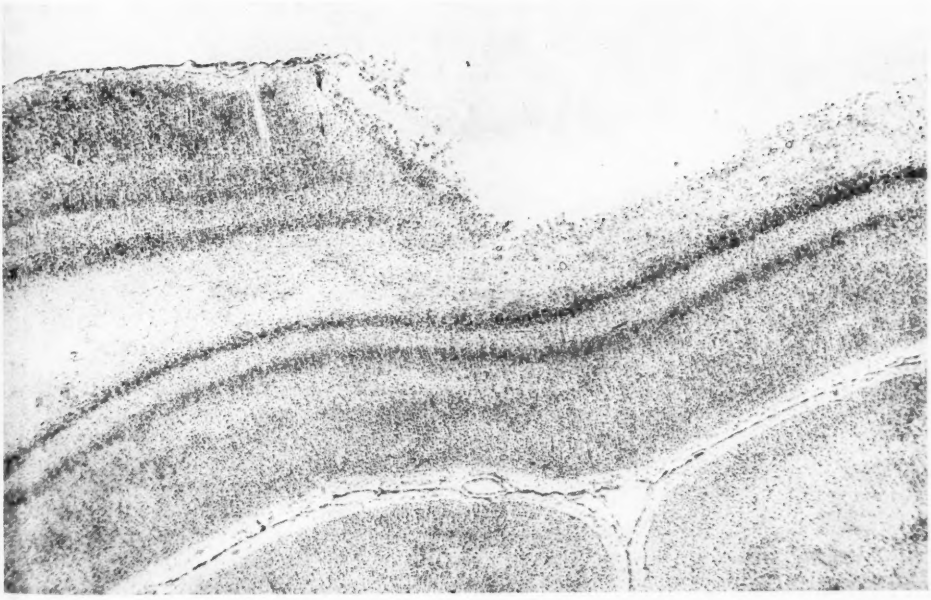

Fig. 2.

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EXPLANATION OF PLATE I

Fig. 1. ×10. Fig. 2. ×27.

Microphotographs of a lesion in the visual area showing complete separation and absorption of the involved cortex, the steep edge of the lesion corresponding to the line of vascular obliteration, and the shallow block-shaped medullary lesion which has the same shape as the area of cortical destruction. The section shown in Fig. 1 is stained by the nitroprusside-benzidene method to demonstrate the vascular pattern, whilst that illustrated in Fig. 2 is stained with Borrel's methylene blue.

THE OCCURRENCE OF WANDERING CELLS IN CULTURES OF NERVOUS TISSUE *IN VITRO*, AND THE CHANGES IN THEIR FORM IN VARIOUS MEDIA[1]

By W. JABLONSKI and H. MEYER

From the Anatomical Institute, The University of Turin.
Director: Prof. G. Levi

During our experiments on cultivation of nervous tissue *in vitro* over a long period with the Fischer-Parker method,[2] we noticed that after 4 days or even earlier there emigrated many free cells from the explanted tissue into the coagulum. In the beginning these cells had the typical form of macrophages.[3] Their number increases during the following days, and simultaneously their shape changes. The fat vanishes, the cells become smaller and elongated, and send out pseudopodia. Relatively frequent are star-shaped cells, with three long, ramifying processes. After 3 or 4 weeks' cultivation with the Fischer-Parker technique, the explant is surrounded by a wide area of these cells (Fig. 2).

In the beginning, we supposed that these cells are specific elements of the nervous tissue. This assumption did not come true.

We investigated the morphological changes of these cells and their relations to the state of the medium in cultures of various nervous tissues (retina, brain, spinal ganglia), and they have been compared with the monocytes of the blood *in vitro* and with the free cells in cultures of some non-nervous tissues (heart, "Anlage" of cross-striated muscles).

For the description of morphology and the typical behaviour of the cells we take as a paradigm the free cells in cultures of the retina. This material accords very favourable conditions for studying the free cells, as the retina *in vitro* grows slowly, and there emigrate only a few nerve fibres, but neither fibroblasts nor epithelial cells, and later on, too, the free cells only migrate for any considerable distance from the explant. We generally used embryos from the 14th to the 20th day of incubation.

After 24 hours, cells of the typical form of macrophages form a halo round

[1] With the aid of the Rockefeller Foundation.

[2] *J. Anat.* vol. LXXII. The cultures have been cultivated in Carrel flasks type A. The solid phase consists of 0·5 c.c. plasm, 1·0 Ringer solution and 1 drop diluted embryonal extract. They are washed three times a week during 2 hours with Ringer solution, and then remain in the thermostat for 4 hours with heparinized plasm. They did not need being transplanted and could be observed up to 4 months.

[3] In the literature on explantation, cells of this kind are referred to as macrophages, histiocytes or clasmatocytes. Usually, the round, vacuolized cells with undulating membrane are described as macrophages, the long, ramified forms as histiocytes. There are, however, transitional forms between the macrophages and the histiocytes.

the explant. They contain fat droplets and have an undulating membrane. From the 4th or 5th day on, the most peripherally situated cells begin to change their form. They ramify, decrease, form chains, and eventually lose the fat droplets. Later on they elongate, their cytoplasm becomes hyaline, and finally they are like clasmatocytes in every detail. Typical mitoses are frequent and can be watched under the microscope. Many different forms of cells can be found, there being transitional forms between all of them (Fig. 1).

There is no upper limit to the extension of the area of free cells except the one given by the size of the coagulum. The phenomenon of the non-confluence of neighbouring areas of free cells as described by Carrel & Ebeling (1926) has been very distinct in our cultures too.

In fixed preparations, small fat droplets are seen near the nucleus. After vital staining there appear numerous small neutral red vacuoles occupying the whole central part of the cell, whereas they are absent in the processes. The filiform appendices frequently spread out at the end, thus forming small hyaline membranes similar to those of the macrophages. Similar structures have been described by Moellendorff. Sometimes, especially in zones of liquefaction, the cells flatten out at the cover slip and become very large (Fig. 4).

We watched these free cells up to 3 months. They remained in good state up to this time.

Simultaneously, we cultivated explants of the retina in Carrel flasks according to the usual technique,[1] with the intention of testing the influence of the medium on form and properties of these cells. In this case only very few macrophages appear, and do not change their shape nor become histiocytes.

In cultures of spinal ganglia, cultivated with the technique of Fischer and Parker, in the first days, besides the flattening of the explant, emigration of fibroblast-like cells and outgrowth of nerve fibres occurs. Only after 2 or 3 days there appear some rounded macrophages, full of fat droplets, in the explanted tissue. Very soon their number increases, and they wander in the direction of the periphery. They generally are nearer to the surface of the coagulum than the other cells.

After about 8 days these cells have migrated to the margin of the explanted tissue and beyond it in the coagulum. They then lose the fat droplets, become elongated, and as a whole transform in the same sense as the macrophages in cultures of the retina (Figs. 3, 5). These transformations can be followed, leaving the flasks for several days in the inverted microscope. Sometimes it has been seen that a great many of these cells adhere to foreign bodies, bits of cotton and cracks in the mica. In this case they retain their macrophage-like form.

According to what has been said about cultures of the retina, in cultures of spinal ganglia cultivated with the usual technique the number of free cells

[1] Solid phase: 0·5 c.c. plasm, 1·0 Ringer solution and 1 drop of diluted embryonal extract. Fluid phase: 0·5 c.c. Ringer and three drops of diluted embryonal extract.

is considerably less, they do not lose the fat droplets, and retain the form of macrophages. A few cells can become a little more ramified, but the very elongated cells with hyaline protoplasm are not found, and after a short time the free cells die.

Similar things happen in brain cultures. As soon as the macrophages have reached the periphery of the zone of outgrowth (composed by epithelial cells and nerve fibres), in these cultures the free cells alter their form in the same way as in the cultures of the other nervous tissues mentioned above (Fig. 6). Cultures of the brain frequently liquefy the coagulum, and in the zone of liquefaction the free cells are flattened out and adhere to the cover slip.

With the intention of comparing the free cells in cultures of nervous tissues and in cultures of non-nervous tissues, we have cultivated some non-nervous tissues by the method of Fischer and Parker; i.e. the monocytes of the blood, the "Anlage" of cross-striated muscle, and the ventricle of the heart.

There exists a detailed description of the morphology of the monocytes of the blood cultivated in presence of embryonal extract by Carrel & Ebeling (1926). They mostly have the shape of macrophages, i.e. they are rounded cells with undulating membrane, with many vacuoles staining with neutral red, and very refractory fat droplets. The ramified, elongated forms which we have described above occur but rarely. Ephrussi (1930) has pointed out that the macrophages become histiocytes if cultivated in absence of embryonal extract. This we could confirm, and we could see that the monocytes of the blood, if cultivated by the Fischer-Parker technique, after some days become elongated and lose the vacuoles, and that then their cytoplasm becomes hyaline. The cells are then extremely similar to the free cells in cultures of nervous tissue. Maybe they generally are not quite so elongated as the latter, and have a more irregular shape. But that could be due to a more intense amoeboid movement. There is no difference in their reaction to neutral red from that of the free cells of the retina.

Cells similar to those in cultures of the monocytes of the blood sometimes occur in cultures of the ventricle of the heart and of the "Anlage" of cross-striated muscles, but they sometimes are absent. Here, again, if they are cultivated in absence of embryonal extract, they become typical histiocytes. They usually are less numerous in cultures of non-nervous tissues than in cultures of brain or retina.

DISCUSSION

There can be no doubt that the free cells we described in cultures of the retina, spinal ganglia and brain, and other tissues, correspond to the clasmatocytes (histiocytes of Aschoff, polyblasts of Maximow). In our cultures they appear in the form of clasmatocytes as well as of macrophages, according to the quality of the medium. The histiocyte form seems to be very resistant: we have watched these cells up to 3 months.

There are not many reports on the free cells in cultures of nervous tissue. After a short reference to the cells described by Veratti (1919), Costero (1930) and

Wells & Carmichael (1930) have been the first ones to study them thoroughly. Costero describes these cells in hanging drop cultures of the brain of chicken embryos from the 15th to the 20th day of incubation as free amoeboid cells of irregular form. They are pronouncedly phagocytic and become round after phagocytosis. They stain with the Hortega method. Costero compared the form of these cells *in vitro* with the form of cells in the organism, and found analogies with the microglia cells and the "Koernchen-Zellen". But cells *in vitro* should be compared as to their form with cells *in vitro* only.

Wells and Carmichael describe three types of cells in cultures of brain in presence of embryo juice; they interpret them as microglia cells. There are cells with one long process, small cells with two to three processes, and round cells similar to the microglia cells of the adult brain (?).

In the laboratory of Lewis, v. Mihalik (1932) has investigated the nature of the free wandering cells which occur in cultures of the brain. According to v. Mihalik the wandering cells appearing in cultures of chicken embryos of 3–21 days' incubation are histiocytes (macrophages). They are identical with the "Gluge" cells (third element of Cajal). There can be no doubt, according to v. Mihalik, that the wandering cells he describes are identical with the cells which Costero describes, interpreting them as microglia cells, and which, Veratti, Kapel (1927, 1929), Wells & Carmichael and Verne (1930) have described. They are macrophages. He cultivated in the same culture liver and brain, and skin and brain, and did not find any difference between the cells emigrating from the different tissues. Our results completely agree with the results of v. Mihalik; i.e. the free cells emigrating from cultures of brain and retina correspond to the cells which, according to their actual form, are defined as histiocytes (clasmatocytes) or macrophages. As a new fact, it appears from our results that the cells, which in the beginning have the form of macrophages, if cultivated in absence of embryonal extract, lose the accumulated fat and become small longish and ramified histiocytes.

The cells we found in our cultures are completely identical with the ones usually called histiocytes or macrophages. We do not stress the fact that there are differences in form. Maximow, many years ago, pointed out that the polyblasts, which according to him derive from the resting wandering cells and, at least partly, are identical with the macrophages of Metschnikoff, can assume various forms.

With our technique the histiocytes are, up to a certain point, similar to the microglia cells. But there is no evidence that the microglia cells are in fact typical histiocytes. From the retina of the embryo of 6 days, which does not yet contain any vessels, no histiocytes are found to emigrate; maybe this fact is in favour of what the majority of authors assume, i.e. that they derive from the macrocytes of the blood. But, at this stage, the retina does not yet contain microglia cells either. Further researches with this favourable material perhaps might give a definite proof of the origin of the microglia cells from the histiocytes.

SUMMARY

We have found, in cultures of retina and brain, after a few days' cultivation by the method of Fischer and Parker, macrophages transforming into small histiocytes. They are amoeboid, contain neutral red vacuoles, and often are seen to divide mitotically. These cells retain the form of histiocytes for months. Their number in our cultures is excessively large, and increases in course of time. In cultures by the classical method of Carrel their number is much smaller, because they do not multiply, at least not to this degree, and they retain the typical form of macrophages without transforming into histiocytes. We cannot regard them as specific components of nervous tissue, because in cultures *in vitro* of other organs (spinal ganglia, heart), too, the same cells appear.

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EXPLANATION OF PLATE I

Fig. 1. Culture 172. Retina of chicken embryo of 15 days incubated *in vitro* for 22 days. Elongated and star-shaped cells. Photo *in vivo*, ×75.

Fig. 2. Culture Mg. 10. Retina of chicken embryo of 17 days cultivated *in vitro* for 8 days. Vital stain with neutral red. The explant is surrounded by a wide area of free cells. Photo *in vivo*, ×50.

Fig. 3. Culture Mg. 4 of a spinal ganglia of a chicken embryo of 15 days cultivated *in vitro* for 15 days. Elongated and star-shaped histiocytes. Vital stain with neutral red. Photo *in vivo*.

Fig. 4. Culture 1852 of retina of a chicken embryo of 15 days incubated *in vitro* for 11 weeks. Elongated and star-shaped cells and many flattened to the cover-slip adherent cells. Vital stain with neutral red. Photo *in vivo*.

Fig. 5. Culture 175. Spinal ganglion of a chicken embryo of 11 days incubated *in vitro* for 17 days. Elongated and star-shaped cells. Photo *in vivo*, ×50.

Fig. 6. Culture Mg. 17. Brain of a chicken embryo of 15 days incubated *in vitro* for 24 days. Elongated and star-shaped cells. Photo *in vivo*, ×75.

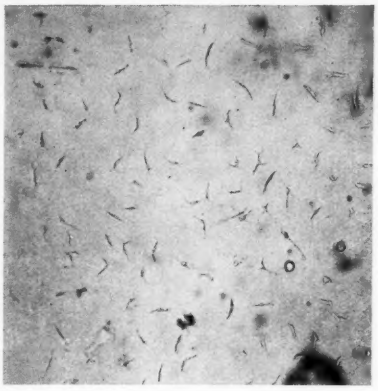


Fig. 1.

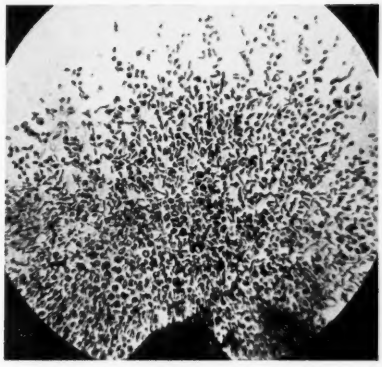
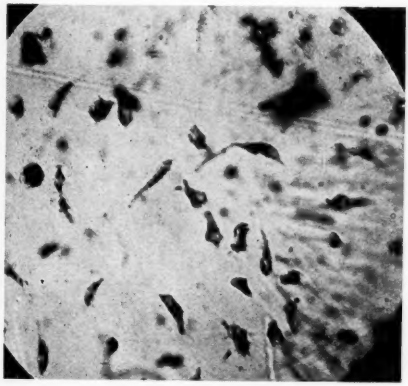


Fig. 2.



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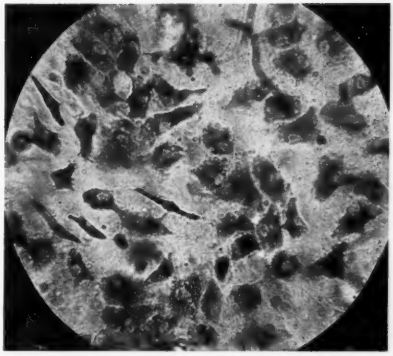

Fig. 4.

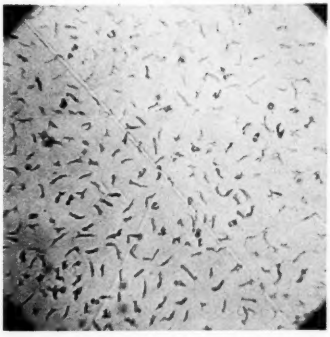

Fig. 5.

Fig. 6.

JABLONSKY AND MEYER—NERVOUS TISSUE *IN VITRO*

UNIV. OF MICH.

THE EFFECT OF SEX-HORMONES ON THE BULBO-URETHRAL GLANDS OF RHESUS MONKEYS

By OLIVE E. AYKROYD and S. ZUCKERMAN
From the Department of Human Anatomy, Oxford

The available records, which have been recently reviewed by Heller (1932), suggest that the size and structure of Cowper's glands are dependent upon the secretions of the testes. In most species the glands involute after castration, while they show seasonal changes in size and functional capacity in animals which reproduce in only a restricted part of the year (Griffiths, 1889; Wells, 1935). Furthermore, testicular hormone can prevent castration atrophy of the glands if its administration is begun at the time of the gonadectomy, or it can restore them to normal after castration changes have occurred (rat, Heller, 1930, 1932; ground-squirrel, Wells, 1935). Totally different effects are produced by oestrogenic hormone. According to Heller (1932) such stimulation has no effect on the gland in castrated male rats, but leads to involution, which in all respects resembles castration atrophy, in normal males. Heller relates this finding to the hypothesis advanced by Moore & Price (1932) to explain gonadal-hypophyseal relationships. On the other hand, Wells (1936) reports that the injection of oestrin (human placental extract) into both normal and castrated ground-squirrels during the season of low sexual activity causes enlargement of Cowper's gland and metaplastic stratification (with desquamation of superficial cornified cells) of both the smaller collecting ducts and the main duct of the gland. In addition, the secretory epithelium grows in height and granules accumulate at "the distal pole of epithelial cytoplasm". The secretion found in the lumina of the tubules of animals treated in this way is also atypical in being interspersed with sloughed epithelial cells and leucocytes. The same treatment given during the breeding season to normal males leads to shrinkage of the gland and to loss of its contained secretion. It also leads, as during the non-breeding season, to stratification of both the smaller collecting ducts and the main duct. The latter response is even better marked in the mouse. Burrows (1935, 1937) has recently reported that as a result of oestrogenic stimulation the bulbo-urethral glands in this animal become smaller owing to the loss of cytoplasm in the secretory cells, and that the epithelium of the whole organ undergoes squamous metaplasia if the treatment is continued long enough. The metaplasia begins at the urethral opening of the main duct and extends peripherally. As a result, cystic dilatation and suppuration may occur in the glands.

In the rhesus monkey prolonged oestrogenic stimulation does not lead to metaplastic changes in the glandular acini of Cowper's glands (Zuckerman, 1938). The following study was therefore undertaken in order to examine more

closely the nature of the responses of the gland to both androgenic and oestrogenic stimulation. Some parallel observations were made on the responses of Bartholin's glands in the female of the species.

MATERIAL

Transverse sections of Cowper's glands of the normal rhesus monkeys (*Macaca mulatta*) listed in Tables I and II were studied and used as controls. The testes of these animals were examined histologically in order to determine whether or not spermatogenesis was in progress at the time of autopsy. Ages have been estimated according to the tables provided by Schultz for body-weight-dentition relationships (1933, 1935), and for convenience, animals whose estimated ages were no greater than 36 months (Table I), and whose testes on histological examination proved to be completely immature, have been separated from those which were older (Table II).

Table I. *Cowper's gland in immature rhesus monkeys (estimated age not greater than 36 months)*

No.	Estimated age (months)	Per cent acinar tissue	Epithelial height (μ)
395	1	56	13
112	18	51	16
118	19	59	17
125	20	57	18
38	24	60	19
39	28	40	15
1	30	35	15
164	34	49	16
158	34	42	14
140	36	43	14
Mean	—	49·2 ± 2·8	15·8 ± 0·4

Table II. *Cowper's glands in pubertal, adolescent and mature rhesus monkeys (estimated age over 36 months)*

No.	Estimated age (months)	Condition of testes	Per cent acinar tissue	Epithelial height (μ)
163	40	Immature type, no spermatogenesis	57	14
182	44	Immature type, no spermatogenesis	51	15
157	46	Immature type, no spermatogenesis	67	14
90	50	Immature type, no spermatogenesis	56	15
150	60+	Adolescent type, no spermatogenesis	65	15
127	60+	Spermatogenesis	48	11
149	90+	Spermatogenesis	55	16
Mean	—		57·1 ± 2·6	14·5 ± 0·4

Tables III, IV, V and VI give details about immature male rhesus monkeys, whose ages were estimated to be no more than 36 months, which were respectively treated (*a*) with male hormone, (*b*) with oestrone, (*c*) with combinations of these two hormones and (*d*) with combinations of oestrone and progesterone. None of these animals was sexually mature, and histological examination in all cases revealed an immature condition of the testes.

Table III. *Cowper's gland in immature monkeys (estimated age not greater than 36 months) after injection of male hormone*

No.	Estimated age (months)	Nature of preparation	Total amount injected (mg.)	Days injected	Per cent acinar tissue	Epithelial height (μ)
313	20	Androsterone	100	10	62	19
318	24	Androsterone	55	22	47	18
319	24	Androsterone	110	22	36	19
67	24	Androstanediol	60	12	65	18
327	24	Androstanediol	100	20	46	18
47	24	Androstanediol	140	28	60	18
239	16	Testosterone propionate	200	14	49	19
240	16	Testosterone propionate	200	14	58	19
256	24	Testosterone propionate	350	13	71	21
250	24	Testosterone propionate	400	14	63	20
260	24	Testosterone propionate	400	14	61	20
259	28	Testosterone propionate	400	14	62	20
Mean	—	—	—	—	56·7 ± 2·1	19·1 ± 0·2

Table IV. *Cowper's gland in immature rhesus monkeys (estimated age not greater than 36 months) after injection of oestrone*

No.	Estimated age (months)	Amount of oestrone daily (γ)	No. of days	Per cent acinar tissue	Epithelial height (μ)
310	15	5000	6	44	22
304	36	3000	6	61	20
314	15	200	7	33	19
258	24	100	14	49	20
36	28	50	14	67	19
257	28	100	14	61	21
43	30	50	14	42	14
124	30	100	14	48	17
24	36	50	14	52	18
79	30	100	15	56	17
148	32	100	15	39	19
315	30	1000	16	50	20
324	24	200	22	48	17
16	18	200	28	39	18
101	30	100	62	43	15
120	33	2·5	365	44	12
115	28	100	368	42	15
Mean	—	—	—	48·2 ± 1·8	17·9 ± 0·5

Table V. *Cowper's gland in immature rhesus monkeys (estimated age not greater than 36 months) after injection of oestrone and male hormone*

No.	Estimated age (months)	Days injected	Oestrone injected daily (γ)	Male hormone preparation	Total male hormone injected (mg.)	Per cent acinar tissue	Epithelial height (μ)
320	24	16	200	Androsterone	40	50	20
329	24	14	100	Androstanediol	70	54	18
330	25	14	100	Androstanediol	70	59	22
49	28	14	100	Androstanediol	70	69	19
177	32	14	100	Testosterone	35	65	20
179	28	14	100	Testosterone	35	64	20
170	36	14	100	Testosterone	35	56	20
162	24	14	100	Testosterone propionate	50	65	17
161	32	14	100	Testosterone propionate	25	63	18
176	34	14	100	Testosterone propionate	10	63	21
Mean	—	—	—	—	—	60·0 ± 2·0	19·5 ± 0·4

Table VI. *Cowper's gland in immature rhesus monkeys (estimated age not greater than 36 months) after injection of oestrone and progesterone*

No.	Estimated age (months)	Days injected	Oestrone injected daily (γ)	Progesterone injected daily (mg.)	Per cent acinar tissue	Epithelial height (μ)
14	20	14	200	0·20	60	19
15	20	14	200	0·20	59	18
17	20	28	200	0·20	62	17
22	30	14	50	0·20	54	17
19	34	14	200	0·50	65	17
41	35	14	50	0·75	60	18
Mean	—	—	—	—	59·9 ± 2·3	17·4 ± 0·3

OBSERVATIONS

Methods

Cowper's glands. The bulbo-urethral glands in the male rhesus are discrete organs and can be easily dissected from the surrounding tissue. Owing however to the fact that in the pursuit of a routine most of the specimens used in the present study had already been embedded and sectioned, it was impossible except in six selected cases to obtain the dimensions of the whole organ. Where this was done the maximum length, breadth and thickness were measured from serial sections.

All specimens were sectioned at 10μ and stained either with Mayer's haemalum and eosin or with haematoxylin and eosin. A selected few were also stained with mucicarmine or Toluidin blue.

The proportion of acinar to stromal tissue was measured on tracings of projections of the microscopic preparations. The projections were made at a linear magnification of × 420, and the part of each gland which was traced was that which fell, without any selection, into an arbitrarily constructed, irregularly quadrilateral frame measuring 54·4 cm.^{2} The outlines of the acini were traced in pencil, and their total area was measured by means of a planimeter and expressed as a percentage of the whole area traced. Each specimen was treated twice in this way. The results are given in Table VII, and by inspection it can be seen that the random choice of the area projected has no significant influence on the observed relation of acinar to stromal tissue. The percentages given in Tables I–VI are the means of each pair of observations made on the different specimens.

Table VII. *Reliability of estimations of epithelial height and ratio of acinar to stromal tissue*

Class	No. of specimens	Mean per cent acinar tissue		Mean epithelial height (μ)	
		1st reading	2nd reading	1st reading	2nd reading
Normal, under 36 months	10	48·7 ± 3·4	49·7 ± 3·3	15·8 ± 0·6	15·7 ± 0·5
Normal, over 36 months	7	57·1 ± 3·6	57·1 ± 4·1	14·4 ± 0·6	14·6 ± 0·5
Male hormone	12	54·1 ± 2·9	59·3 ± 3·3	19·1 ± 0·3	19·1 ± 0·3
Oestrone	17	47·9 ± 2·9	48·6 ± 2·2	18·0 ± 0·7	17·7 ± 0·6
Oestrone + male hormone	10	60·1 ± 2·2	60·0 ± 3·5	19·5 ± 0·5	19·5 ± 0·5
Oestrone + progesterone	6	58·3 ± 2·8	61·5 ± 3·7	17·3 ± 0·4	17·5 ± 0·4

The number of complete circular or elliptical sections of separate alveoli within the areas that were traced was counted. A separate count was made of the number of alveoli which were only partly within the projection-frame, and this count was again divided into (*a*) those alveoli whose outlines constituted more than a semicircle, and (*b*) those alveoli which projected into the frame for less than a semicircle. Three of the former category were regarded as forming two, and three of the latter category as forming one complete acinus. In this way an approximate idea was obtained of the number of acini per unit area in the different specimens.

The mean height of ten acinar cells in each specimen was measured in μ with a micrometer eyepiece. Five of the ten cells measured were picked at random from the peripheral and five from the central region of each specimen. The mean epithelial height of each gland was calculated a second time in the same way in order to obviate any possibility of unconscious selection. The high order of reliability of the observations is shown in the last two columns of Table VII.

Bartholin's glands. The bulbo-urethral glands are difficult to see at autopsy in the female monkey, and only thirteen pairs of glands which had fortuitously been included in serial sections of the distal part of the vagina were studied. Six of the thirteen were from normal animals and seven from spayed females which had received either oestrone alone or varying combinations of oestrone and testosterone propionate. Epithelial height and the proportion of glandular to stromal tissue were calculated in the same way as in the male monkey.

RESULTS

Cowper's glands

General.

Normal growth (Tables I and II). The form of the secretory epithelium does not appear to alter significantly with age, the cells during all phases of growth usually being columnar and having relatively small, basally-disposed nuclei (Plate I). The height of the epithelium appears, however, to increase in maturity (see below), and at the same time the relative amount of fibro-areolar stroma decreases. The glands grow very gradually in gross size during prepubertal life, and apparently very rapidly as maturity is reached. The glands of the fully mature rhesus monkey are many times larger than those of the immature animal.

The smaller collecting ducts are lined by either one or two layers of cuboidal or, more rarely, columnar epithelium which in places may have a cytological character similar to the secretory cells of the acini (Pl. II, fig. 8). The epithelium of the larger ducts may also be plicated, while the main collecting duct is typically lined by a single layer of cuboidal epithelium. Both the smaller and larger collecting ducts are frequently distended.

In general it may be said that there is much less gross variation with age

in the form and character of Cowper's glands than occurs in the other accessory reproductive organs.

Castration. The only involution that had occurred in the bulbo-urethral glands of a mature male rhesus which had been castrated five months before autopsy was limited in extent and restricted to the peripheral part of the gland (Pl. II, fig. 5). Little, if any, change had occurred in the ratio of acinar to stromal tissue or in the height of most of the secretory cells (Pl. I, fig. 4). It would thus seem that Cowper's glands in the mature rhesus monkey are not dissimilar from those of the mature guinea-pig, which, according to Barrington (1913) show little regression in the first year after castration. They are very unlike those of the rat (Heller, 1932), which rapidly show marked castration changes.

Influence of male hormone (Table III). Apart from increasing the height of the secretory epithelium and the ratio of acinar to stromal tissue (see below) male hormone, within the limits of the experiments summarized in Table III, causes no obvious changes in the structure of Cowper's glands in the rhesus monkey. Whether or not a gross change in size occurs could not be established directly with the few data that were available. Consideration of the changes that occur in the ratio of stromal to acinar tissue indicates, however, that the gland grows appreciably and rapidly under the influence of androgenic stimulation (see below).

Influence of oestrone (Table IV). Cowper's glands do not appear to grow under the influence of prolonged oestrogenic stimulation, nor does there seem to be any change in the ratio of acinar to stromal tissue (see below). On the other hand the height of the secretory epithelium in the immature monkey is significantly increased. The general character of the gland, with one main exception in the series of seventeen studied, was not different from that in the control series (Table I). The exceptional animal was 115, which had been injected with 100γ of oestrone daily for 368 days. The glands of this animal appeared to be somewhat involuted relative to the control specimens, the acini often being smaller than usual and more widely separated by stroma. The secretory epithelium was also lower. The glands of 101 and 120 were very similar in these respects, and it is significant that these three animals had received the longest treatment.

No metaplastic changes were observed in the acini (Pl. II, fig. 6), the smaller collecting ducts, or the main ducts (Pl. II, figs. 7 and 8) of any of the animals—two of which, as indicated in Table IV, had been injected for a year. Cowper's glands in the monkey would thus appear to differ significantly from those of the mouse and ground-squirrel in their reactions to oestrogenic stimulation.

In two specimens of this series collections of lymphocytes were observed within the glands. Similar "nodules" occur elsewhere in the urogenital tract of both normal monkeys, and monkeys that have been injected with oestrone (Sandys and Zuckerman, 1938).

Influence of oestrone and male hormone and of oestrone and progesterone (Tables V and VI). Apart from changes in gross size, epithelial height, and the ratio of acinar to stromal tissue, no obvious changes occur in Cowper's glands as a result of the simultaneous administration of oestrone and male hormone, or of oestrone and progesterone.

The ratio of stromal to acinar tissue. The significance of the differences between the mean values for per cent acinar tissue in the different classes (Tables I–VI) has been estimated by means of Fisher's *t* test (Fisher, 1934) and the results are collected in Table VIII. The following differences are obviously significant, the greater ratio of acinar to stromal tissue in each case being put second:

The difference between

(i) the immature normal controls and the immature monkeys that had been injected with male hormone;

(ii) the immature normal controls and the immature monkeys that had been injected with both oestrone and male hormone;

(iii) the immature normal controls and the immature monkeys that had been injected with oestrone and progesterone;

(iv) the animals that had been injected with oestrone and those that had been injected with both oestrone and male hormone;

(v) the animals that had been injected with oestrone and those that had been given male hormone.

Table VIII. *The significance of the differences of the mean values for per cent acinar tissue and epithelial height. Those values of t which are in italics represent significant differences.*

Classes compared	Degrees of freedom	Per cent acinar tissue *t*	Epithelial height *t*
Immature normals (I.N.) with "mature" normals	32	2·24	2·05
I.N. with oestrone gp.	52	0·33	*4·99*
I.N. with male hormone gp.	42	*2·39*	*7·52*
I.N. with oestrone + male hormone gp.	38	*3·53*	*6·72*
I.N. with oestrone + progesterone gp.	30	*3·11*	*2·77*
Oestrone gp. with oestrone + male hormone gp.	52	*4·25*	*2·40*
Oestrone gp. with male hormone gp.	56	*3·13*	2·00
Oestrone + male hormone gp. with male hormone gp.	42	1·11	0·99

The value of *t* for the comparison between the immature normal group and the group of normals estimated to be older than 36 months, though high, does not indicate a significant difference. Further data would be necessary to decide whether or not these two classes do differ significantly; *a priori* it would be supposed that they do. In the way the older group is at present constituted it mostly includes animals whose testes were in an immature condition, and unfortunately further material from fully mature animals is at present unavailable.

The remaining values of t are so low that it is highly improbable that significant differences exist in the ratio of acinar to stromal tissue between the classes from which the groups considered are drawn (immature normals as compared with corresponding animals that have been injected with oestrone; animals that have been injected with male hormone alone as compared with animals that have been given both male hormone and oestrone). These results thus indicate that oestrone, in the amounts injected, did not act synergically with male hormone, as it is sometimes believed to do. On the other hand, since oestrone appears to have no effect of its own on the ratio of acinar to stromal tissue, the fact that it has such an effect when combined with progesterone either implies that it acts synergically with that hormone on Cowper's glands, or that progesterone itself can influence the acinar tissue of the gland. Similarly, the results of the experiments detailed in Table V indicate that oestrone, in the amounts in which it was given, did not inhibit the action on Cowper's glands of the male hormone which was simultaneously administered. It is of interest, therefore, that in the same experiments the effects which oestrone normally has on the prostate and seminal vesicles were in general inhibited by the male hormone (Zuckerman & Parkes, 1936; Zuckerman, 1936). On the other hand the oestrogenic effects of oestrone on the prostate were not inhibited by the progesterone.

Estimation of the number of acini per unit area (p. 139) shows that this factor is approximately constant in the different classes of animals studied. Consequently it follows that an increase in the ratio of acinar to stromal tissue in the main implies an increase in the size of the individual acini and not an increase in their number. It also therefore follows that in those instances where the relative amount of acinar tissue has increased as a result of experimental treatment, the gross size of the glands has also increased, for the possibility that the absolute amount of stromal tissue would simultaneously decrease is remote.

Epithelial height. The significance of the differences between the mean values for epithelial height in the different classes of animals studied (Tables I–VI) has also been estimated by Fisher's t test (see Table VIII). It will be seen that epithelial height has increased significantly under each experimental regimen, including that of oestrogenic stimulation, but that the epithelial height in the immature normal group (Table I) is not significantly different from that in the older normal animals (Table II). *A priori* it would be expected that epithelial height would be greater in sexually mature animals, and the present result is to be explained, as in the case of the relative amount of acinar tissue (p. 141), as being due to the fact that only two fully mature animals are represented in our older group of animals.

It is of interest that, while high, the value of t shows that the mean epithelial height in animals treated with oestrone is not significantly different from that of animals treated with male hormone, whereas there is a significant difference between the former and the mean epithelial height in animals which

had been treated with both oestrone and male hormone. This suggests that under the conditions of the present experiments male hormone is able to enhance the stimulating effect which oestrone has on the epithelial cells. On the other hand the fact that there is no significant difference in epithelial height between animals which have been treated with male hormone alone and animals which have received both oestrone and male hormone suggests that oestrone, under the conditions of the present experiments, does not act as a "synergist" to male hormone on the epithelium of Cowper's glands.

In so far as oestrone increases epithelial height, Cowper's glands in the immature rhesus monkey react in the same way as do those of the ground-squirrel during the period of low sexual activity (Wells, 1936) and unlike those of the mouse (Burrows, 1937). The stimulating effect of oestrone on the epithelium appears, however, to diminish as oestrogenic stimulation continues, for with one exception (43), the lowest epithelium met with in our series of seventeen animals (Table IV) was in the three animals which received the longest treatment (101, 120, 115).

It may be noted that epithelial height and the ratio of acinar to stromal tissue usually, but not always, alter in the same way under the influence of sex-hormone stimulation.

Bartholin's glands

The series of thirteen glands studied was neither sufficiently extensive nor representative enough to indicate more than that in general the bulbo-urethral glands in the female rhesus monkey react in much the same way as Cowper's glands to sex-hormone stimulation.

CONCLUSIONS AND SUMMARY

Cowper's glands in the fully mature rhesus monkey are much larger, show a greater ratio of acinar to stromal tissue, and have taller secretory cells than in the immature animal. The glands involute relatively slowly after castration.

The glands increase in total size under the influence of androgenic stimulation. The same treatment induces a rapid increase in epithelial height and in the ratio of acinar to stromal tissue.

Under the influence of oestrogenic stimulation the secretory cells of Cowper's glands in immature monkeys increase significantly in height, but as the treatment proceeds they appear to return to their normal size. The ratio of acinar to stromal tissue does not appear to alter, nor is there any evidence of an increase in the gross size of the glands at any stage of the treatment.

Oestrogenic treatment does not cause metaplasia in either the ducts or acini of the glands (maximum experimental period one year) and in this respect the monkey differs from mice and ground-squirrels. Corresponding differences are shown in the prostatic reactions of these animals to oestrogenic stimulation.

Oestrone does not enhance, under the conditions of the present experiments, the effect of male hormone on either the ratio of acinar tissue or on the height of the secretory epithelium. On the other hand male hormone enhances the effect which oestrone has on epithelial height. Moreover while oestrone alone does not have any effect on the ratio of acinar to stromal tissue, it has such an effect when combined with progesterone.

We wish to express our best thanks to Dr R. B. Fisher for his help in the statistical analysis of our data. The material used in the present study was obtained from monkeys bought with the aid of a grant to S. Z. from the Medical Research Council. The work was also assisted by a grant to S. Z. from the Nuffield Medical Committee, Oxford. The hormones used were very generously provided by Dr Karl Miescher of Messrs Ciba Ltd, and Dr Tausk of Messrs Organon.

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EXPLANATION OF PLATES I AND II

Plate I. Epithelium of Cowper's gland. ×500

Fig. 1. OM 395. New-born male.
Fig. 2. OM 1. Prepubertal male.
Fig. 3. OM 149. Fully mature male.
Fig. 4. OM 209. Castrated fully mature male.

Plate II. Cowper's gland

Fig. 5. OM 209. Castrated fully mature male, showing regions of involution. ×56.
Fig. 6. OM 115. Pubertal male, after a year's treatment with oestrone. ×500.
Fig. 7. OM 115. Main ducts of the glands. Normal form and disposition. ×35.
Fig. 8. OM 115. Main duct of gland, showing absence of metaplasia after a year's treatment with oestrone. The cells in the centre are mucoid in character. ×550.

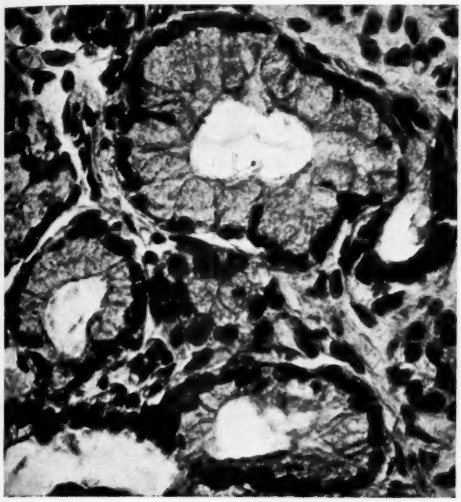
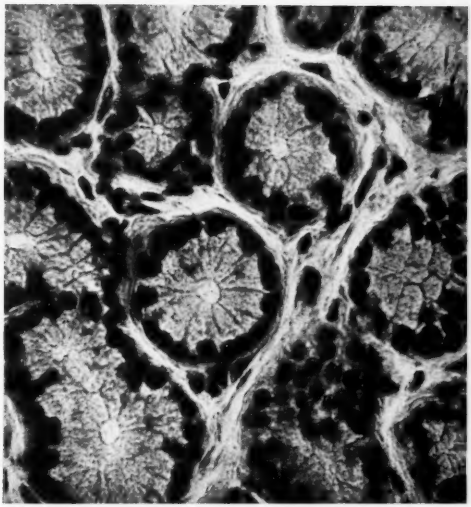


Fig. 1.



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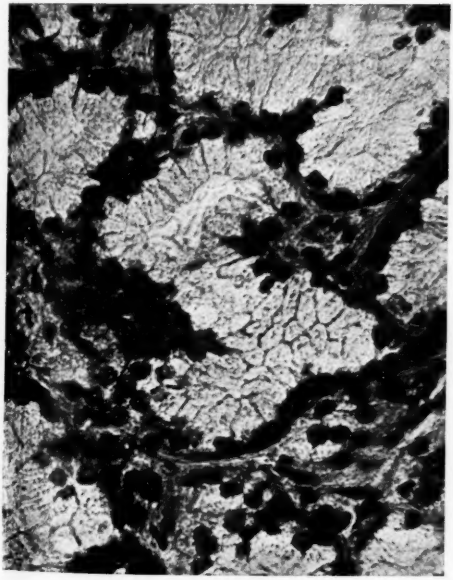

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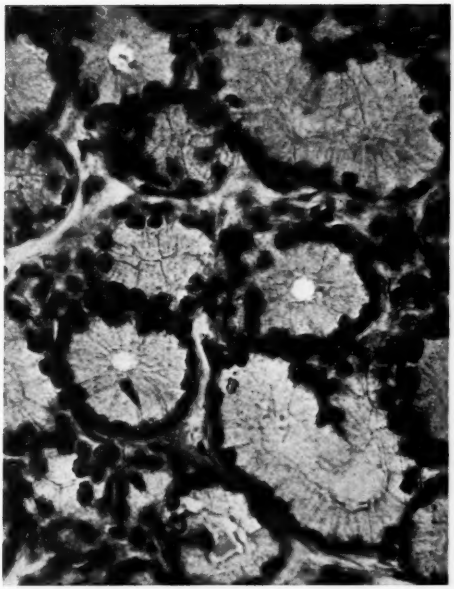

Fig. 4.

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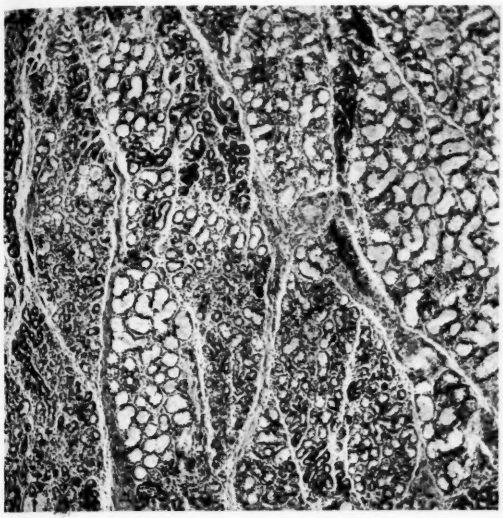

Fig. 5.

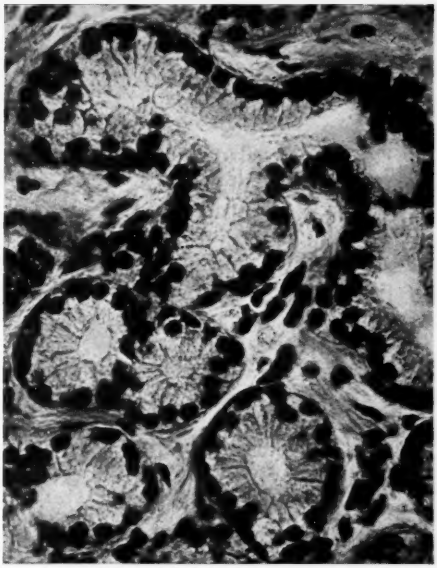

Fig. 6.

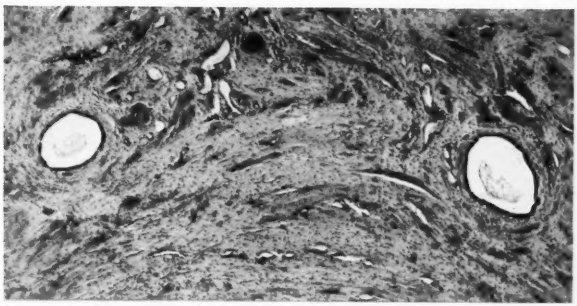

Fig. 7.

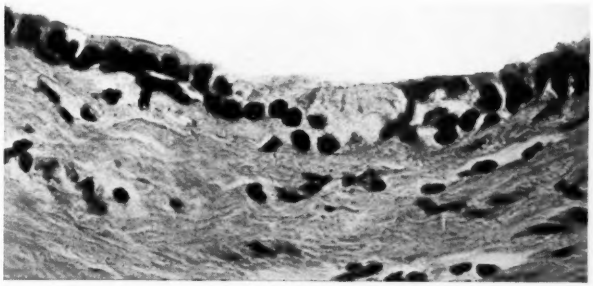

Fig. 8.

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EXPERIMENTS ON THE DEVELOPMENT OF THE PRONEPHRIC DUCT

By R. J. O'CONNOR
Department of Anatomy, University College, London

INTRODUCTION

In *Amblystoma tigrinum*[1] and *Triton taeniatus* the rudiment of the nephric system first appears as a thickening of the somatopleure immediately beneath the ectoderm at the ventrolateral border of certain somites at the anterior end of the embryo. The limits of this thickening are at first difficult to define but in both species it is seen soon after the closure of the neural folds in the region from the third to the seventh somites. At a little later stage of development the thickening becomes more precisely defined and being immediately beneath the ectoderm gives rise to a ridge visible from the exterior. In succeeding stages of development the nephric rudiment extends in a caudal direction, maintains throughout its position at the ventrolateral border of the somites, and eventually reaches the cloaca as a solid column of cells, there being a corresponding extension of the ridge caused by it. The pronephros is formed from the rudiment in the vicinity of the third and fourth somites while the more caudal portion becomes canalized to form the pronephric duct.

The mode of this caudal extension has been a matter of controversy. There are two possibilities; first the rudiment may extend caudally by its own independent growth, or secondly it may be extended by an addition to it from each successive segment. Mollier (1890) could not decide between the two possibilities but was inclined to support the first. Field (1891) however concluded that the extension of the rudiment was due to a proliferation of the somatopleure *in situ*, his evidence in *A. punctatum* being the similarity of yolk granules in the nephric rudiment and adjacent somatopleure. Field's conclusion has been accepted by more modern writers and has been repeated by Goodrich (1930) and Felix (1906), while Burlend (1931) supports the conclusion on comparative grounds. Brauer (1902) described an independent caudal growth of the nephric rudiment in the rare Gymnophonia but in accepting this Burlend states it to be atypical. Therefore it appears that opinion and evidence favour the view that the portion of the nephric rudiment giving rise to the pronephric duct is formed *in situ* by a contribution from each individual segment.

As a means of studying the mode of formation of the pronephric duct experiments have been performed on *T. taeniatus* and *A. tigrinum* in which

[1] I am indebted to Miss R. M. Renton of the Zoology Department, University College, for her kindness in supplying me with the spawn of *Amblystoma tigrinum*.

localized portions of the developing nephric system have been stained with Nile blue sulphate in the living embryo.

TECHNIQUE

The technique was adapted from Stone's (1931) modification of Vogt's vital staining methods and Nile blue sulphate was applied to the embryos by means of small pieces of agar soaked in the dye. A 2 % solution of agar was poured on to a glass plate and evaporation produced thin sheets of a rubbery consistency. After drying these for 2 or 3 days they were soaked in a strong solution of Nile blue sulphate and became impregnated with the dye. The nephric rudiment was exposed by dissecting back a flap of overlying ectoderm using a hair loop and glass needle according to the technique of Spemann and the exposed rudiment was stained directly by the application of a piece of agar corresponding in size to the exposed area. The agar was held in position by means of a fragment of a cover-slip and removed 2 hr. later, the ectodermal flap being allowed to fall back into position. In addition to an intense staining of the exposed tissues stain was also taken up by the ectodermal flap and by the ectoderm at the rim of the wound and this staining of the ectoderm served as a useful means of localizing the stained region in serial sections. The operation wound healed in 3 hr. and subsequent development was always normal.

Experiments were performed at all stages from the first appearance of the nephric rudiment up to its junction with the cloaca. The object was to stain the caudal limit of the rudiment and this could be fairly accurately determined by following the caudal lengthening of the external ridge due to it and developmental tables are available to check such observations. It was found that the caudal limit of the rudiment was always stained when an exposing wound, the width of two somites, was made over the caudal end of the ridge. In Pl. I, fig. 1, is shown an embryo of *T. taeniatus* at stage 25 (Glaesner, 1925) stained in this way. The photograph was taken 5 hr. after operation when healing was complete.

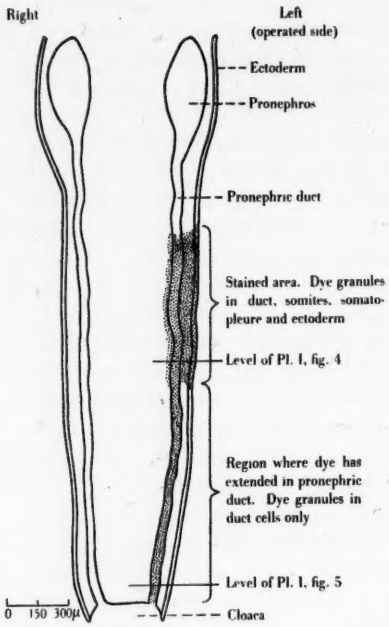
RESULTS OF STAINING EXPERIMENTS

In a high percentage of cases when the caudal limit of the nephric rudiment was stained in the manner described the dye spread in a definite and constant fashion. First there was a general increase in the size of the stained area with a simultaneous decrease in the intensity of staining; no significance is attached to this as it was obviously due to the growth of the embryo. In addition however there occurred a spread of the dye as a narrow streak leading to the cloaca and situated at the ventrolateral border of the somites. The width of this streak of dye was consistent with that of the nephric rudiment, the dye was beneath the ectoderm, and in its extension from the stained area to the cloaca it kept pace with the extension of the ridge of the nephric rudiment of the opposite side. The presumption was therefore that the streak was due to the presence of dye in the developing pronephric duct. This could be confirmed

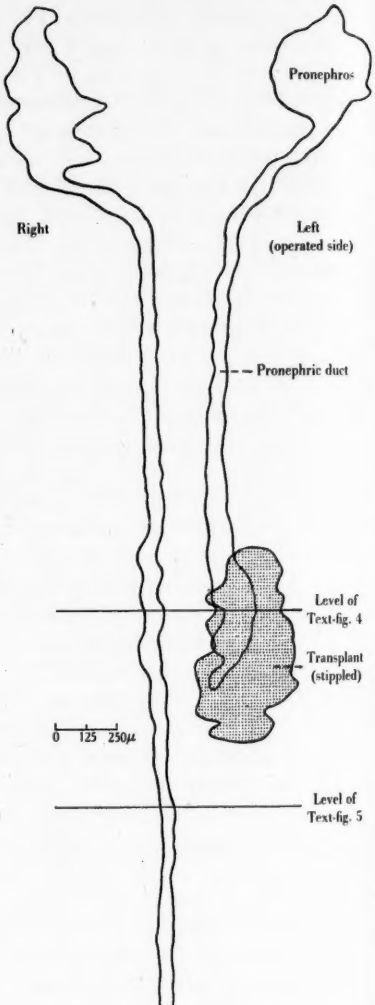
by dissection and a blue pronephric duct demonstrated leading from the stained area to the cloaca. The characteristics of the extension of the dye are shown in Pl. I, figs. 2 and 3. Fig. 2 is of an embryo of *T. taeniatus* which had been operated on 2 days previously at stage 22. The caudal limit of the developing nephric rudiment was stained at the level of somites 8 and 9 on the left side. The photograph was taken at stage 26–27 and the streak-like extension of the dye is seen. Actually the dye almost reached the cloaca but in the posterior third of the animal it was too faint to photograph. In Fig. 3 a similar result is seen in *A. tigrinum* which was stained, in the region of somites 8–11 on the left side, two days before when the embryo was at stage 25 (Harrison).[1] The photograph was taken at stage 35 and the streak of dye is seen to reach the cloaca. The actual site of staining is somewhat obscured by the pigmentation of the ectoderm.

External examination and simple dissection left no doubt that these blue streaks were due to dye in the pronephric duct but did not exclude the possibility that it might be in a partially formed lumen. Therefore embryos were examined by serial section. The preservation of Nile blue sulphate during sectioning is a matter of some difficulty as the dye is soluble in alcohol. Stone's method of adding 0·1% phosphomolybdic acid to all alcoholic solutions solved the difficulty and it was found an advantage to make up the alcohol-phosphomolybdic acid solutions immediately before use. Rapid dehydration did not interfere with the production of good sections and specimens were left 10–15 min. in the alcoholic solutions, and sections were mostly cut at a thickness of 10 μ. In the sections the dye appeared as blue granules and its distribution could be determined. The result of a typical experiment is shown in Text-fig. 1. This diagram was obtained by drawing each section with the aid of the projection microscope and then measuring the width of the structures represented and their distance from the midline; these measurements were then plotted to scale on squared paper and areas where dye granules were present represented by stippling. The experiment represented is one on *A. tigrinum*. Two days previously the nephric rudiment was stained ventral to somites 8 and 9 at stage 25. At the time of sectioning the embryo was a stage 35 and a streak of dye was present leading from the stained area to the cloaca. In the sections the stained area was recognized by the presence of dye granules in the ectoderm and in this region blue granules were seen in the adjacent somite, in the somatopleure and in the cells of the pronephric duct (Pl. I, fig. 4). Caudal to the stained area the dye was confined to the cells of the pronephric duct, there being none in the adjacent structures. This is seen in Pl. I, fig. 5 which is two or three sections from the cranial border of the cloaca. In this section the few granules seen in the ectoderm, myotome and somatopleure are due to the pigment of the embryo and microscopically are easily distinguishable from the Nile blue sulphate granules in the pronephric duct. At all levels in the

[1] Harrison's numbered stages refer to the development of *A. punctatum* but the development of *A. tigrinum* is sufficiently similar to justify the use of Harrison's stages.


Right
Left
(operated side)
Ectoderm
Pronephros
Pronephric duct
Stained area. Dye granules in duct, somites, somatopleure and ectoderm
Level of Pl. I, fig. 4
Region where dye has extended in pronephric duct. Dye granules in duct cells only
Level of Pl. I, fig. 5
0 150 300μ
Cloaca

Text-fig. 1.



Text-fig. 3.

Text-fig. 1. Distribution of Nile blue sulphate (shown as stippling) at stage 35. Left nephric rudiment stained ventral to somites 7–9 two days previously when embryo was at stage 25. Levels of figs. 4 and 5, Plate I, indicated.

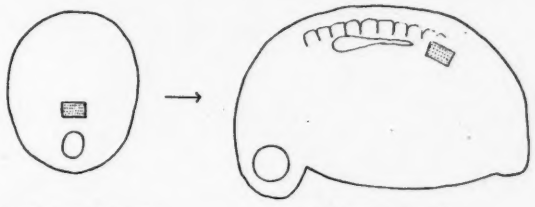
Text-fig. 3. Nephric system in *A. tigrinum* at stage 39–40. Nine days previously transplantation performed as shown in text-fig. 2. Levels of text-figs. 4 and 5 indicated.

pronephric duct the dye was found in the cells alone there being none in any lumen present.

It is clear that the dye in the cells of the caudal portion of the pronephric duct could not be derived from the unstained adjacent cells and the only possible source could have been the dye placed in the caudal limit of the nephric rudiment which must therefore be the origin of the more caudal portions subsequently developed.

TRANSPLANTATION EXPERIMENTS

The vital staining experiments described above furnish evidence that the nephric rudiment extends caudally by its own independent growth and this evidence is supplemented by experiments where an obstruction is grafted in the path of its caudal extension. Such experiments were performed on both *A. tigrinum* and *T. taeniatus* and the hosts chosen from stages where the nephric rudiment is confined to the more anterior segments. A transplant, taken from another embryo, was placed at the ventrolateral border of somites beyond the caudal limit of the rudiment, and Text-fig. 2 illustrates such an experiment.



T. taeniatus. Stage 11. *A. tigrinum.* Stage 23.

Text-fig. 2.

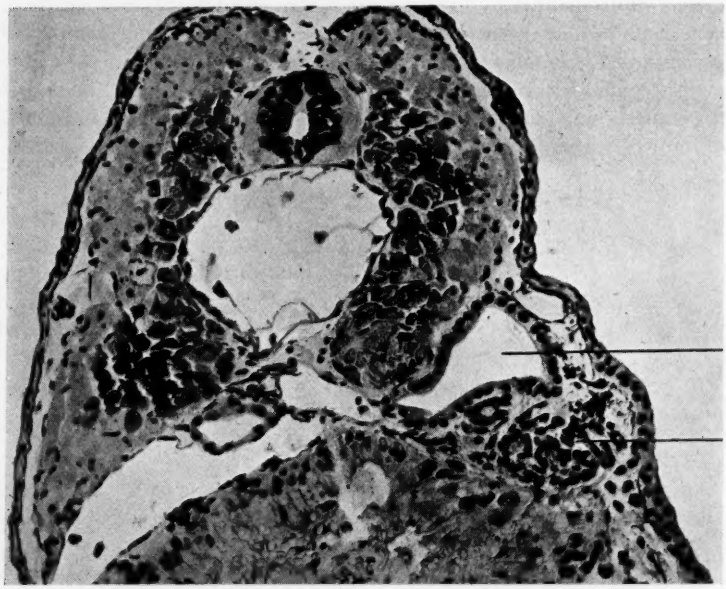
A portion of the presumptive neural area from the gastrula of *T. taeniatus* was transplanted at the ventrolateral border of somites 9–10 in *A. tigrinum* at stage 23 when the nephric rudiment had not reached beyond the seventh or eighth somite. The host embryo was sectioned at the ninth day at stage 39–40 and the arrangement of nephric system and transplant is represented in Text-fig. 3 which is a diagram made in the same way as Text-fig. 1. The non-operated side has undergone a normal development. On the operated side the transplant has differentiated into nervous tissue, and on this side the pronephric duct becomes dilated as it comes into the region of the transplant (Text-fig. 4). It then abruptly ceases and caudal to the transplant there is no sign of its formation (Text-fig. 5).

It was immaterial what sort of tissue was used for the transplant and the above result was obtained in both *A. tigrinum* and *T. taeniatus* using nervous tissue, phronephros and cloaca as the transplant. Development was followed in these cases up to 30 days and in every case there was absence of the duct caudal to the graft. It is apparent that there is no contribution to the forma-

tion of the pronephric duct from the more posterior segments and so in normal development it must be entirely formed by a growth caudally of the nephric rudiment.

CONSIDERATION OF A SERIES OF VITAL STAINING EXPERIMENTS

If the conclusion that the pronephric duct is formed by an independent caudal growth of the nephric rudiment be correct then the following results would be expected from vital staining experiments. Firstly when the caudal limit of the rudiment is stained at any stage prior to its union with the cloaca



Text-fig. 4. Transverse section of *A. tigrinum* at the level shown in text-fig. 3. Cells of transplant (*c.t.*) and dilated left pronephric duct (*l.d.*) are seen. × 120.

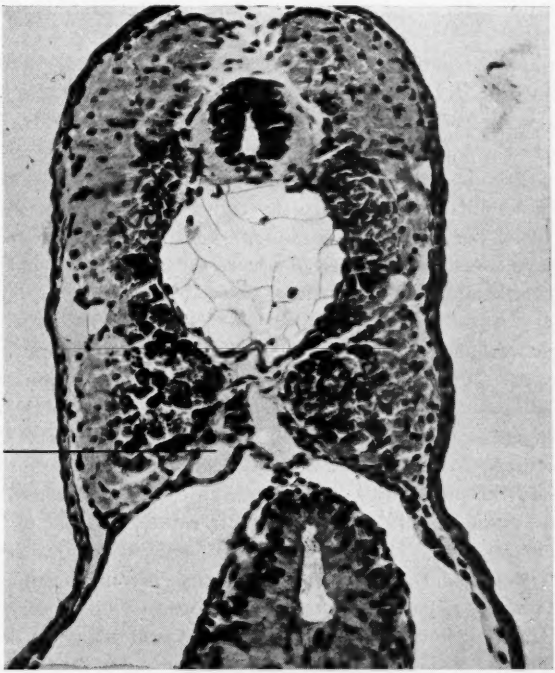
the dye should extend towards this structure in the cells of the pronephric duct; that is a positive result. Secondly, when the stain is placed some distance cranial or caudal to this point in the line of the development of the rudiment there should be no extension of the dye; that is a negative result. One hundred and twenty-one experiments on *T. taeniatus* and *A. tigrinum* can be divided into two such groups:

(1) Stain in the caudal limit of the rudiment: expected positive.

(2) Stain cranial or caudal to the limit: expected negative.

Table I. *Showing the number of cases where expected result was obtained in vital staining experiments*

	Cases	Positive	Negative	Expected result obtained (1) Cases	Expected result obtained (2) Per cent.
A. Tigrinum:					
(1) Expected positive	24	24	0	24	100
(2) Expected negative	16	3	13	13	81
Total for *A. tigrinum*	40	27	13	37	92
T. taeniatus:					
(1) Expected positive	41	37	4	37	90
(2) Expected negative	40	5	35	35	87
Total for *T. taeniatus*	81	42	39	72	89
Total for whole series	121	69	52	109	90



Text-fig. 5. Transverse section of *A. tigrinum* at level shown in text-fig. 3. Absence of left pronephric duct. (Right pronephric duct, *r.d.*) × 120.

In Table I the results of this series of experiments are shown and it will be seen that in each group for both species the expected result is obtained in over 80 % of cases and in the whole series in 90 %.

THE QUESTION OF A SEGMENTAL ORIGIN OF THE NEPHRIC SYSTEM

Although Mollier was not able to determine the mode of extension of the nephric rudiment he came to the conclusion that the portion of the rudiment ventral to somites 3–6 in *T. alpestris* was formed *in situ* as a proliferation of the somatopleure and so was derived segmentally, and in the original rudiment Field, in *A. punctatum*, and Glaesner, in *T. taeniatus*, record some signs of segmentation. Further evidence of a segmental origin of the original nephric rudiment was obtained from vital staining experiments on *A. tigrinum* and *T. taeniatus*. In both species the site of the future nephric rudiment was stained in the neurula stage before its appearance. There are no visible somites at this stage of development and so the stain was placed by guesswork and its exact position determined when later the somites and nephric rudiment appeared.

Two groups of experiments on *T. taeniatus* can be considered, similar results being obtained in *A. tigrinum*.

(1) Dye was placed in the vicinity of somites 3 and 4 and confined to these segments. As the nephric rudiments appeared that portion of it in segments 3 and 4 was seen to be stained, but no other portion, the dye remaining where it was placed and not extending into the rudiment of adjacent fifth and sixth segments. That portion of the rudiment ventral to somites 5 and 6 must therefore have come from a different source to that portion ventral to somites 3 and 4. Examination at later stages showed the dye to be confined to the pronephros.

(2) Dye was placed in segments 5 and 6 in the vicinity of the somitic region. The dye in this case could be traced into the portion of the nephric rudiment in association with these segments but not into the more cranial portion. This latter portion, therefore, could not have received a contribution from segments 5 and 6. Examination by serial section or by dissection at later stages showed the pronephros to be free from dye granules, but in some cases there was an extension of dye into the more caudal portions of the rudiment.

In experiments such as these, performed without the guidance of nephric rudiment or somites, accurate placing of the dye is a matter of some difficulty and the fate of dye placed in a single segment cannot be given. However, it is clear that the nephric rudiment of segments 3 and 4 have a different origin to that of somites 5 and 6, and when this evidence is added to the morphological findings it is possible to support the conclusion that the original nephric rudiment is derived in a segmental manner, each of the segments 3–6 and possibly 7 contributing to its formation.

In the second group of experiments where stain was traced into the rudiment of segments 5 and 6 the dye in some cases extended toward the cloaca in the nephric rudiment. The significance of this finding was investigated by a further series of experiments. These were performed on *A. tigrinum* at stage 22

and on *T. taeniatus* at stage 19. At these stages the nephric rudiment becomes clearly demarcated at the ventrolateral border of somites 4–7 with the production of an external ridge. This and the appearance of somites enables the nephric rudiment to be stained with a fair degree of accuracy. In each species the experiments were of two types:

(1) Where the rudiment of segments 3 and 4 was stained.

(2) Where the rudiment of segments 5–7 was stained.

Table II. *Results of staining nephric rudiment when it is first clearly demarcated* (A. tigrinum *St.* 22; T. taeniatus *St.* 19)

	Cases	Extension	Percentage
Stain in segments 3–4:			
(1) *A. tigrinum* (St. 22)	8	1	12
(2) *T. taeniatus* (St. 19)	15	2	14
Total	23	3	13
Stain in segments 5–7:			
(1) *A. tigrinum* (St. 22)	9	9	100
(2) *T. taeniatus* (St. 19)	20	20	100
Total	29	29	100

The results of these experiments are set out in Table II. In both species staining the rudiment in segments 3 and 4 produces less than 15 % of cases where the dye extended into the caudal portions of the rudiment and in the three cases where it did occur the extension was only for a distance of two or three somites and is readily ascribable to an inaccurate placing of the stain. In the negative cases dissection or serial section showed the dye to be confined to the pronephros. On the other hand, in the cases of staining the rudiment of segments 5–7, extension of the dye occurred in 100 % of cases. In these experiments no dye could be traced into the pronephros but it could be traced into the pronephric duct. In all cases it extended more than half the length of the duct and in the majority could be traced as far caudally as the cloaca. Therefore the final conclusion is that the first rudiment of the nephric system is derived in a segmental manner in the region of the third to seventh somites. The cranial portion under somites 3 and 4 forms the pronephros while the more caudal portion under somites 5–7 gives rise to the pronephric duct by its own independent caudal growth.

SUMMARY

1. A technique is described for vitally staining, with Nile blue sulphate, localized portions of the developing nephric system in *A. tigrinum* and *T. taeniatus*.

2. It is shown that the pronephric duct in these species is formed by an independent caudal growth of the first formed nephric rudiment. The evidence for this conclusion is:

(*a*) When Nile blue sulphate is placed in the caudal limit of the nephric

rudiment at any stage prior to its union to the cloaca the dye can be found in the pronephric duct cells from the stained area to the cloaca.

(*b*) When the caudal growth of the nephric rudiment is prevented by a suitably placed transplant there is no formation of the pronephric duct caudal to the transplant.

3. Evidence derived from vital staining experiments shows that the first formed nephric rudiment is derived in a segmental manner from the third to seventh segments and that while the cranial portion forms the pronephros the caudal portion grows independently toward the cloaca to give rise to the pronephric duct.

ACKNOWLEDGEMENT

The writer is indebted to Prof. H. H. Woollard for facilities to carry out this work, and for much helpful advice and criticism during the preparation of this paper. Part of the expense of this research was borne by a grant from the Research Fund of the University of London.

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DESCRIPTION OF PLATE I

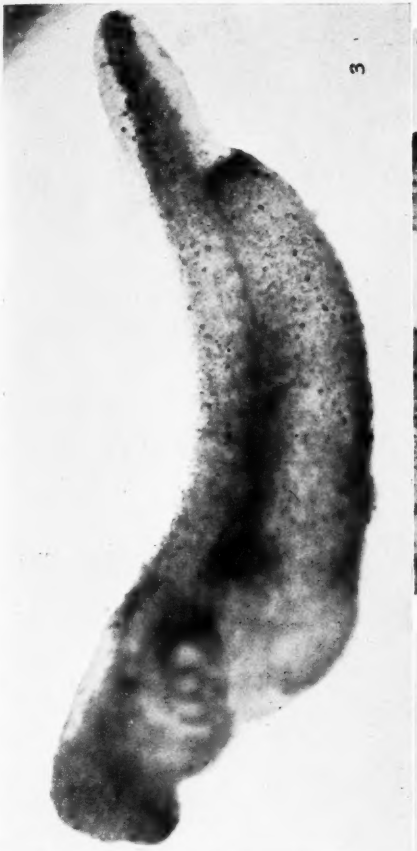
Fig. 1. Embryo of *T. taeniatus* at stage 22. Left nephric rudiment stained five hours previously with Nile blue sulphate. × 25.

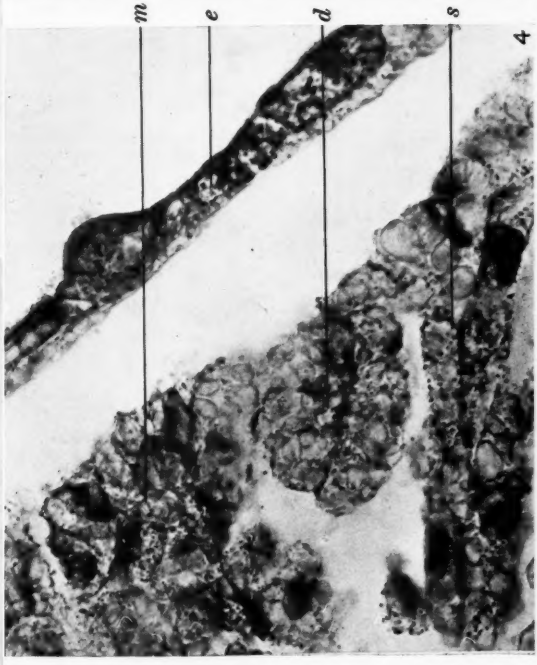
Fig. 2. Embryo of *T. taeniatus* at stage 26–27. Two days previously at stage 22 nephric rudiment stained with Nile blue sulphate ventral to 8–9 somites. Extension of dye as a streak at ventrolateral border of somites. × 25.

Fig. 3. Embryo of *A. tigrinum* at stage 35. Two days previously at stage 23 nephric rudiment stained with Nile blue sulphate ventral to somites 8–9. Extension of dye as a streak at the ventrolateral border of somites. × 25.

Fig. 4. Transverse section through left pronephric duct of *A. tigrinum* at level shown in Fig. 4. Granules of Nile blue sulphate in cells of duct, myotome, somatopleure and ectoderm. (*d*, duct; *m*, myotome; *s*, somatopleure; *e*, ectoderm.) × 620.

Fig. 5. Transverse section through left pronephric duct of *A. tigrinum* at level shown in Fig. 4. Granules of Nile blue sulphate in cells of duct only. (Lettering as in Fig. 6.) × 620.





UNIV. OF MICH.

A RESURVEY OF THE ANATOMICAL FEATURES OF THE PILTDOWN SKULL WITH SOME OBSERVATIONS ON THE RECENTLY DISCOVERED SWANSCOMBE SKULL. PART I

By SIR ARTHUR KEITH

On 29 June 1935, Mr Alvan Marston, a practising dentist, had the fortune to expose in the 100 ft. terrace of the Thames Valley, at a depth of 24 ft. 3 in. from the surface, an intact and complete occipital bone of a human skull.[1] The site is near Swanscombe, on the south side of the river and nearly two miles above Gravesend. The terrace here is one of the richest sites of palaeolithic implements in all Europe. In the following year, in the same seam, but 7·3 m. distant, he found the left parietal bone of the same skull—also complete. The two bones articulate perfectly. Mr Marston rightly claims that the Swanscombe bones are the only human fossil remains so far discovered in Europe to which a mid-pleistocene date can be assigned with precision and certainty. The implements of the seam are assigned by l'Abbé Breuil to Acheul III.

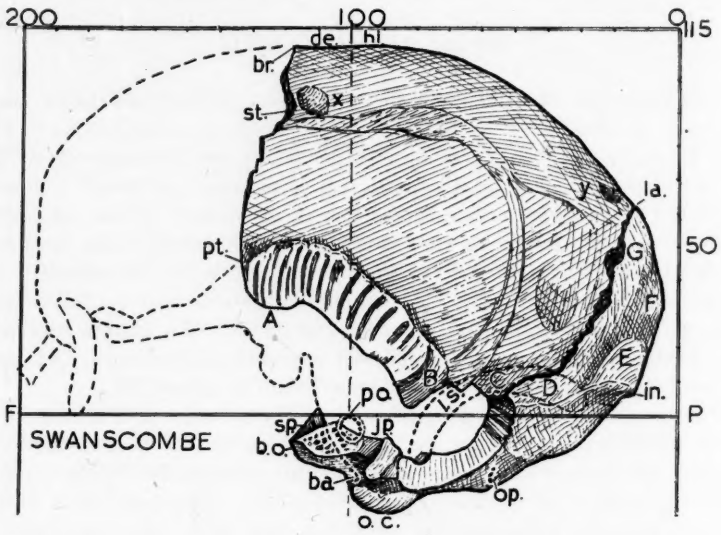
I propose to give a brief description of the Swanscombe skull because, from an anatomist's point of view, Mr Marston's account is not altogether satisfactory. My main reason, however, is this: my examination of the excellent casts which Mr F. O. Barlow has prepared of the Swanscombe bones (and all my drawings and measurements are taken from these casts) convinced me that it had Piltdown affinities. Now one of the chief points in which Sir Arthur Smith Woodward and the late Sir Grafton Elliot Smith and I differed, when the reconstruction of the Piltdown skull was debated a quarter of a century ago, was the manner in which the occipital fragment should be articulated to the rest of the skull. As this relationship is beyond dispute in the Swanscombe skull, I again set up the Piltdown fragments, giving the occipital its Swanscombe relationships. The result was that I had, after six months of repeated endeavour, to modify my original reconstruction very considerably. The nearest approach to the form of the skull I am to describe now is that published in *Essays on the Evolution of Man* (1927) by the late Sir Grafton Elliot Smith. In this, the latest of his reconstructions, Sir Grafton acknowledges the co-operation of Dr John Beattie.

Another reason for my resurvey is this: In recent years two able German anatomists have studied the Piltdown fragments and published very full accounts, Dr H. F. Friederichs in 1932 and Prof. Hans Weinert in 1933. I wished

[1] Mr Marston has given a full account of his discovery and of the skull (*J. R. anthrop. Inst.*, 1937, vol. LXVII, p. 339). References to authors are given according to date of publication.

to ascertain the methods they employed and the results they had obtained, and how such results compared with those published in England a quarter of a century ago. Naturally I was interested in the points wherein they agreed and wherein they differed from the reconstruction I had made in 1913 and published in 1915 (Keith, 1915).

In Text-fig. 1, the Swanscombe bones have been articulated and their left aspect drawn in true profile. They are shown on a plane (F.P.) which is an

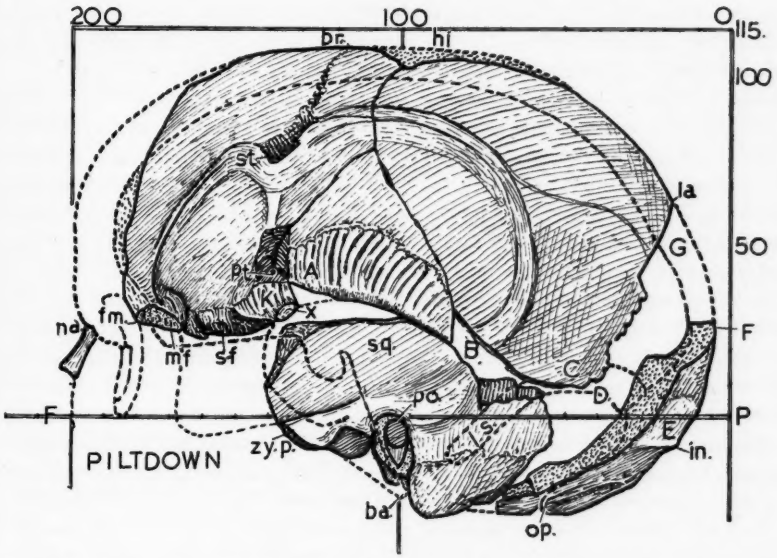


Text-fig. 1. The Swanscombe occipital and parietal bones, articulated, and drawn in true profile and orientated on a plane approaching the Frankfort (F.P.). The framework of lines is 200 mm. long (it extends 100 mm. in front of, and 100 mm. behind, the vertical auricular plane); the upper horizontal line is drawn 115 mm. above the F.P. An outline of the missing frontal is indicated. *A*, lower or squamous margin, behind pterion; *B*, dovetailed part of margin; *C*, on parietal, above asterion; *D*, on lateral sinus and asterionic process of occipital; *E*, *F*, *G*, three markings or ridges on occipital (see text). *ba*, basion; *b.o*, basi-occipital; *br*, bregma; *de*, post-coronal depression; *hi*, highest point of vault (verticon); *in*, inion; *jp*, jugular process; *la*, lambda; *ls*, lateral sinus; *oc*, occipital condyle; *op*, opisthion; *po*, porion; *pt*, pterion; *sp*, septum between halves of sphenoidal sinus; *st*, stephanion; *x*, *y*, excavated areas—due to injury or disease.

approximation to the Frankfort plane. Seeing that in this skull we have neither ear nor orbit, the reader will ask: How do I arrive at this approximation? First, let us take the external auditory meatus. Its relationship to the basilar process of the occipital, a process which is here intact, is very constant in human and anthropoid skulls. It is situated above and behind the basilar process (as shown in Text-fig. 1, *po*), the porion in human skulls being placed usually about 20 mm. vertically above the basion. In the Swanscombe skull the basilar

process is short and is not robust, hence I have placed the porion only 18 mm. above the basion; it may be less, not more.

The best guides to the F.P. in the case of the Swanscombe skull, are the upper and the lower (squamous) borders of the parietal as seen in true profile. It will be observed that I have placed the bregma (*br*) and the highest point (*hi*) of the vault (usually 35 mm. behind the bregma in modern skulls) on the same level. In most anthropoid skulls as in that of *Pithecanthropus* and Rhodesian man the highest point is the bregma, the highest point or *verticon* of the parietal being at a lower level. In Piltdown man the vault (in the F.P. plane)



Text-fig. 2. A drawing of the left aspect of the Piltdown skull—as reconstructed by author in 1938. Lettering as in Text-fig. 1 with following additions: *K*, area on frontal with which the squamosal of temporal articulates; *X*, fragment of great wing of sphenoid still adherent to frontal. *fm*, anterior fronto-malar point, *mf*, malo-frontal articulation; *na*, nasion; *sf*, spheno-frontal articulation; *sq*, squama of temporal; *zy.p*, root of zygoma.

maintains the same level for some distance in front to some distance behind the bregma (Text-fig. 2). In my first series of drawings, I treated the Swanscombe bones as if they were modern, for in their general conformation they resemble many modern bones; I placed the verticon 4 mm. higher than the bregma. When I did so I found the pterion became too low in position to permit a frontal bone to be articulated; the squamous margin assumed an anomalous direction and the plane of the coronal suture, indicated by a line joining bregma and pterion, became too vertical. I inferred that the position of the parietal must be that seen in the Piltdown and in some European skulls (Todd, 1925),

and hence orientated the parieto-occipital with bregma and verticon on the same level. The coronal plane then made an angle of 78° with the F.P. It varies from 70 to 80° in human and anthropoid skulls. Mr Marston, on the other hand, employed the plane of the foramen magnum to obtain an orientation, making this plane to look backwards as well as downwards, at an angle of 12·5° with his horizontal plane. This brings the bregma 10 mm. below the verticon and makes the coronal plane cut the F.P. at an angle of 90°. Such relationships are unknown in any kind of human or anthropoid skull.

In Text-fig. 1, the outline of the missing frontal bone has been copied from that of the Piltdown skull, and is manifestly out of proportion to the parieto-occipital segment. In more primitive skulls the frontal segment makes up about 30 % of the total length. The length of the parieto-occipital segment of the Swanscombe, measured from opisthocranion to pterion, makes 130 mm.; if we add 30 %, namely 55 mm., the total length would have been about 185 mm. The length of the Piltdown skull is greater—190–195 mm. The width of the left half of the Swanscombe skull is 72 mm., giving a maximum width of 144 mm.; the width represents almost 78 % of the length, tending, like the Piltdown skull, towards brachycephaly. It is a low skull, the basi-bregmatic height, measured on the articulated cast, is 128 mm., the height being 88 % of the width. The auricular height shown in Text-fig. 1 is 110 mm. Applying the following Lee-Pearson formula: l. 185 × w. 144 × a.h. 110 × 0·4 + 206—the resultant is a skull capacity of 1370 c.c. Compared with the Piltdown bones those of the Swanscombe skull are moderate in their thickness. In general the parietal varies between 7–9 mm.; at the side, where the width is measured, the Swanscombe parietal is 6·4 mm. thick; at the verticon, 7 mm.; over the occipital pole, where length is measured, 4·6 mm., whereas the Piltdown bones, at corresponding points, measure 10 mm., 11 mm., and 9 mm. If some reduction is made in our estimate on account of the thickness of bone we obtain a capacity of about 1350 c.c. The endocranial cast, taken from my recent reconstruction of the Piltdown skull, displaces 1358 c.c. of water. From my former reconstruction, the endocranial cast measured 1397 c.c.

In Text-fig. 2, I give a drawing of the left profile of my new reconstruction of the Piltdown skull. Its length (glabella-opisthocranion) is 195 mm.[1] The length depends in the first place on the thickness and form we give to the missing forehead. I have moulded the forehead in imitation of the frontal fragment of Piltdown II, discovered by Mr Dawson in 1915. Whereas the forehead of *Pithecanthropus*, *Sinanthropus* and of Neanderthal man are moulded on the lines seen in the frontal of the chimpanzee, gorilla and gibbon, that of *Eoanthropus* followed the form seen in the young orang. The region of the frontal, anterior to the bregma, is almost horizontal and indicates a high forehead; so does the steep ascent of the temporal lines as they leave the zygomatic processes of the frontal. The supraorbital and supraciliary markings of the forehead are similar in many ways to the same parts in juvenile orangs

[1] See also p. 167.

at the age when the second permanent molars erupt. The upper part of the Piltdown face was flat; as reconstructed, the nasion (Text-fig. 2, *na*) is only 15 mm. in front of the anterior fronto-malar point (*fm*), whereas in the Gibraltar skull this distance measures 22 mm.

The other factor which modifies the total length of the Piltdown skull is the position given to the occipital fragment. The impression gathered from Text-fig. 2 is that this fragment has been placed too low down and too far back. This feeling tends to be confirmed when the Swanscombe occiput is superimposed on the Piltdown reconstruction (Text-fig. 25, Part II); the Piltdown fragment is seen to lie behind and below the Swanscombe occipital. On every attempt to give the Piltdown occipital the Swanscombe position, I found, when I uncovered the endocranial cast, that there was a manifest deformation of the cerebellum, which could be remedied only by returning the bone to the position shown in Text-fig. 2. When I pressed the upper part of the occipital forwards, so as to lessen the gap between occipital and parietal (lambda), I reduced the occipital cortex to a most improbable degree.

It may be well to draw attention to the points in which the occipital of these two ancient skulls differ as well as those in which they agree. The distance of the inion from the opisthion in Piltdown is only 46 mm.; in Swanscombe this measures 52 mm.; in Piltdown the inion, in its growth ascent, has not reached the level of the internal inion (Text-fig. 2), in Swanscombe, it has ascended 15 mm. above it. In this respect Swanscombe is the more primitive, the more ape-like; Piltdown, on the other hand, has retained an infantile form. This is a condition we had not expected to find in an early form of humanity; yet it often occurs in the skulls of Bushmen. A high position of the external inion, similar to that seen in the Swanscombe occipital, occurred in the second occipital fragment found by Mr Dawson at Piltdown. In Piltdown the external inion lies 7 mm. below the Frankfort plane; in Swanscombe the same amount above. The difference may be sexual in origin; it does not preclude us from believing that the condition in Piltdown may have evolved into that seen in Swanscombe. We are dealing with a feature which is subject to an exceeding degree of individual variation.

We must note here the curvature of the Swanscombe occipital as seen in profile. We shall see that the external markings of the occipital squama are altogether peculiar (p. 178) and that there are grounds for supposing the squama of the Piltdown to have had a similar form. In Text-fig. 1 I have indicated three of the elevations or curvatures of the occipital squama of the Swanscombe specimen by the letters *E*, *F*, *G*. *E* is seen in Piltdown (Text-fig. 2), part of *F* (Text-fig. 2), but the part bearing the upper curvature has been broken away and is missing. Now the completion of this missing part of the Piltdown occipital has been much discussed; it must have been strongly curved and also high (compare Text-figs. 1, 2). The arc of the Swanscombe occipital measures 115 mm., 52 mm. lying below the inion and 63 mm. above. As reconstructed in Text-fig. 2 the arc of the occipital of Piltdown measures

122 mm., 46 mm. lying below and 76 mm. above the inion. The missing part (Text-fig. 2 *G*) measures 38 mm. The chord (lambda-opisthion) is 97 mm. in the Piltdown occipital and 94 mm. in the Swanscombe. The curvature is also greater in Piltdown (subtense being 31 mm. as against 26 mm. in Swanscombe).

The resemblances of the Piltdown to the Swanscombe cranium, dealt with so far, have been of a general nature. I am now to indicate certain resemblances of a particular kind. The first of these is in the region of the asterion. In modern skulls the lateral sinus generally crosses and grooves the angle of the parietal; the parietal angle serves as an intermediary in carrying the lateral sinus from the occipital to the temporal bones. In the Swanscombe occiput, the lateral sinus passes directly from occipital to temporal—an arrangement which we may speak of as the direct; the ordinary arrangement as the indirect. The direct transit is the usual one in anthropoid skulls and is to be found in a small percentage of modern human crania. With the direct method of transmission there is a special process of the occipital bone which we may name the asterionic angle (see Text-figs. 1, *D*). It fills the angle between parietal and mastoid, being overlapped externally by the angle of the parietal; internally it bears the impression of the lateral sinus, the part on the asterionic process being often demarcated from the rest of the sinus.

With many others I misread the appearances in the asterionic region of the Piltdown parietal. On its inner aspect the margin is raised (Text-fig. 4, p. 163); this raised margin I regarded as only the upper margin of the sinus, the rest having been broken away. There is no doubt that the mode of transmission in Piltdown was, as in Swanscombe, direct; there is a fragment missing from the Piltdown asterionic angle, but the missing part involved only the outer aspect, that which overlapped and covered the asterionic angle of the occipital. In my reconstruction of 1913 I made allowance for the missing angle of the parietal, whereas this angle is really not missing.

The second point of resemblance to impress me was the community in form and shape of the squamous border of the parietal. It is completely preserved in Swanscombe; in Piltdown, a small triangular fragment is missing in front (Text-fig. 2, below *A*); a larger part is missing behind (Fig. 2, *B*); no doubt the dovetailed flange (Text-fig. 1, *B*) was stronger and longer in Piltdown than in Swanscombe. Both have the same arched form. I have searched in vain, during the past years for a squamous articulation which has the conformation and length of striae seen in the Piltdown parietal. The Swanscombe specimen is the nearest approach to it I have seen; the striae, at the widest part of the squamosal articulation, measure 20 mm., whereas those on the Piltdown border are 4 mm. longer. I supposed that the extensive squamosal suture was due to the thickness of the Piltdown parietal; but the moderate thickness in the parietal Swanscombe proves such an explanation to be untenable.

Why should there be this extensive border? The Piltdown temporal bone is bent inwards—under the parietal—more than in any other skull; the temporal muscle exerts its strength across the squamosal suture. It may be the

inward position of the temporal, or its great growth-ascent, that has brought about an extensive squamosal suture.

A third resemblance between Piltdown and Swanscombe is the absence of a spheno-parietal contact; the temporal suture continued forward beyond the pterion, making a temporo-frontal contact, occasionally found in modern skulls, especially in Australian and almost constantly in gorillas and chimpanzees. We cannot tell in the Swanscombe skull how far in front of the coronal suture the squama made contact with the frontal, but in the Piltdown skull its extent is very clear (Text-fig. 2, *K*). In front of this area is that for the great wing of the sphenoid (*sf*); this area passes under *K*, the great wing appearing within the skull (Text-fig. 4). A small fragment of the great wing still remains attached below *K* (Text-fig. 2, *X*). Still further forward in Text-fig. 2 (*mf*) is shown the massive oval contact for the malar or zygomatic, the orbital plate of this bone being very narrow. The malar area of contact is unlike that in any skull known to me—ancient or modern.

Now, in my interpretation of 1913, I supposed that the squamosal contact ended at *A*, and that *K* was part of the sphenoidal contact; as far as I know this has been the interpretation made by all who have sought to reconstruct the Piltdown skull. That the interpretation I am giving is the right one is substantiated by a drawing given in Text-fig. 3 of an ancient Egyptian skull in which there is a temporo-frontal contact. I am greatly indebted to Prof. Wood Jones for placing this specimen at my disposal.

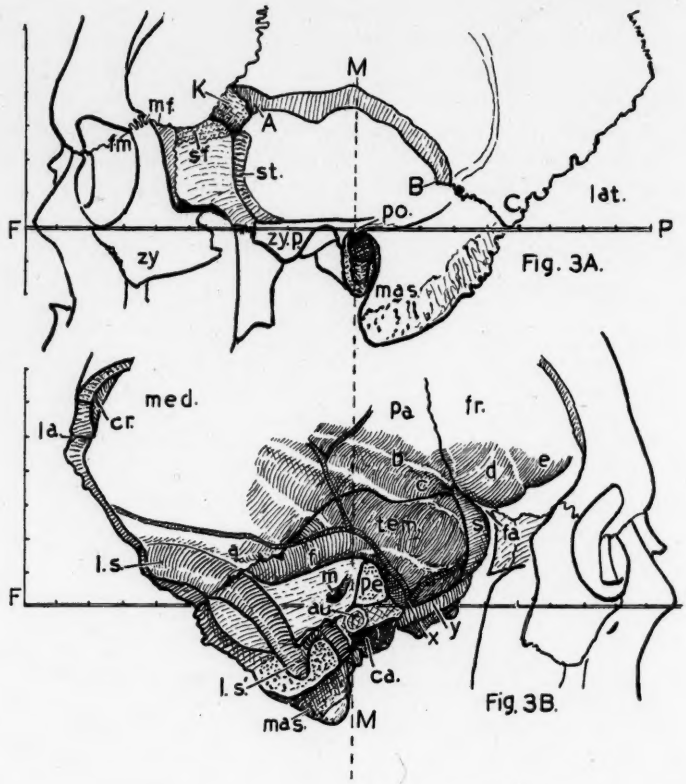
A glance at Text-fig. 3A will remind readers how shallow is the squamosal articulation on the lower margin of the parietal and how weakly developed is the dove-tail process (*B*). The rough eminence on the frontal, for articulation with the squamosal (Text-fig. 3A, *K*), is very similar to the corresponding area in Piltdown (Text-fig. 2, *K*). Further, the articulation of the great wing is also similar (*sf*) but that for the malar or zygomatic is quite different.

There can thus be no doubt that in the Piltdown skull there was a wide temporo-frontal suture and there must have been a similar contact with the missing frontal of the Swanscombe skull. Thus there are three anatomical features, all lying along the lower margin of the parietal which link these two ancient skulls—absence of a groove for the lateral sinus, a wide and peculiar squamosal union and a temporo-frontal contact.

In Text-fig. 30, Part II, is shown the arrangement of sutures of the temporal region in my reconstruction of the Piltdown skull.

We now turn to examine the interior of the pterionic region, for it is in this part of the Piltdown skull we find the most convincing evidence of its very primitive nature. The endocranial cast of this region, as we shall see later, differs from that taken from any other human skull; the third frontal convolution is an exaggeration of the form seen on the endocrania of anthropoid apes.

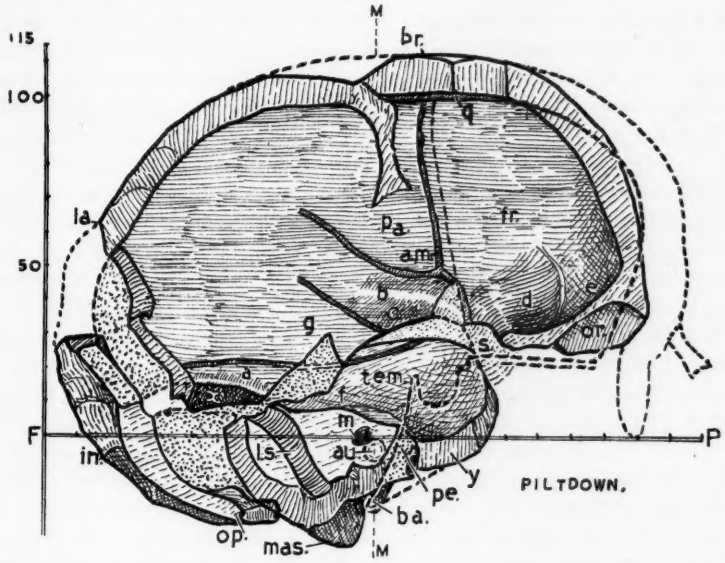
In Text-fig. 3 B is shown the inner pterionic aspect of the Egyptian skull just mentioned. The great wing of the sphenoid appears in the middle fossa;



Text-fig. 3. A. Left lateral aspect of the skull of ancient Egyptian in which the tempora made a wide contact with the frontal. The squamosal has been removed and the great wing of the sphenoid drawn as if it were transparent. The same lettering is used as in Text-fig. 2, with the following additions: *st*, spheno-temporal suture; *zy*, malar bone; *mas*, mastoid process; F.P., Frankfort plane; *MM*, vertical mid-auricular plane at right angles to F.P.

B. Mesial aspect of the same specimen (*a*) on the ridge bounding the upper margin of lateral sinus (*l.s*) on parietal; (*b*) Sylvian ridge; (*c*) impression for first temporal convolution; it passes forwards to the great wing (*s*); (*d*) impression for posterior part of third frontal convolution; (*e*) for anterior part of the same; (*f*) impression for third temporal convolution; *tem*, impression for second temporal convolution, on the inner aspect of squama; *fa*, indicates the falx sylvii, continuous anteriorly with margin of lesser wing; *au*, position of external auditory meatus; *m*, internal auditory meatus; *pe*, apex of petrous; *ca*, carotid foramen; *x*, middle meningeal artery; *y*, sphenoidal margin of temporal; *l.s'*, lateral sinus within mastoid; *mas*, mesial aspect of mastoid process; *la*, lambda; *cr*, internal crest, at hinder end of sagittal suture; *pa*, parietal; *fr*, frontal, with coronal suture between.

it ends above in a pointed extremity which makes contact with the anterior inferior (pterionic) angle of the parietal, for there can be—and often is—a temporo-frontal suture on the outside and a narrow temporo-parietal within, as in this case. The ridge (*b*) which lies in the horizontal branch of the Sylvian (lateral) fissure is clearly marked; it runs forward, becoming prominent and sharp as it ends in the process formed by the frontal, which occupies the stem of the fissure. The sickle-shaped process of bone, formed by parietal as well as frontal, which lies within the stem, I shall speak of as the falx sylvii (Text-fig. 3 B, *fa*). In front of the falx are the impressions for the hinder (*d*) and anterior (*e*) parts of the third frontal convolution.



Text-fig. 4. Interior of left-half of the Piltdown skull. The missing parts are stippled. Lettering as in Text-fig. 3 B, with the following additions: *am*, anterior branch of middle meningeal artery; *q*, groove for longitudinal sinus; *or*, orbital plate of frontal; *g*, area for second or middle temporal gyrus on the parietal; *x*, fractured edge of parietal, at asterion.

On the parietal, below *b*, is seen the impress of the first temporal gyrus; it narrows as it passes forward on the great wing, behind and below the falx; below *c*, is the second temporal gyrus (temp.) wide on the temporal squama and still wider and more complicated as it passes to lie within the parietal bone.

In Text-fig. 4 the interior of the left half of the Piltdown skull is drawn, showing the parts represented in Text-fig. 3 B. The Sylvian ridge (*b*) is wider and flatter than in the Egyptian and, unfortunately, a break occurs, just where this ridge passes forward to join its frontal component of the Sylvian falx. The impression for the first temporal gyrus (*c*) below *b*, is of good width on the

parietal, but undoubtedly it narrowed, just as in the Egyptian, as it passed on to the great wing of the sphenoid. In my original reconstruction I supposed that the great wing was wide as in Neanderthal skulls, hence I separated the squama of the temporal too far from the frontal, so as to give the first temporal gyrus an ample width as it passed to the floor of the middle fossa. Sir Arthur Smith Woodward, on the other hand, brought the temporal almost into juxtaposition with the frontal, diminishing too much the space for the great wing and first temporal gyrus. By reducing the size of the first temporal gyrus in my present reconstruction I have also diminished the height of the vault of the skull.

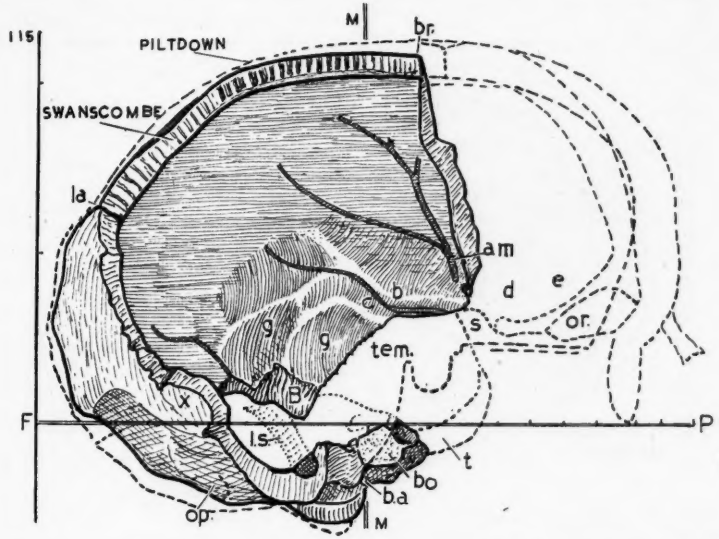
The most striking and anthropoid feature of this region of the Piltdown skull I have not yet mentioned. In Text-fig. 4, the impressions for the two parts of the third frontal gyrus are indicated (*d*, *e*). Behind *d*, the Sylvian margin of the frontal bone, instead of ending in a sharp Sylvian falx, forms a wide, curved elevated surface. This elevation is shaped as in the skull of the gorilla and chimpanzee; in no other human skull has such a conformation been seen. As we shall find, the area of the Sylvian region of the brain is modelled as in anthropoid apes (Pl. I). But whether this is determined by the mechanics of cranial formation or by the innate architecture of the brain, is a problem which is hard to solve. For my own part, I believe, that in early man, as in anthropoid apes, skull growth is dominant and that the developing brain has to accommodate itself to its osseus environment.

Was the arrangement of the Sylvian region in the Swanscombe skull similar to that just described in Piltdown? We have only the markings on the interior of the left parietal to guide us as to what the condition was. In Text-fig. 5 I give a drawing of the articulated Swanscombe bones, seen in right profile. In this drawing the asterionic process of the occipital (*x*) is very noticeable. The markings on the interior of the parietal in the region of the pterion are indicated. The Sylvian ridge (*b*) passes forwards to end on the inturned pterionic angle of the parietal. This angle is missing in the Piltdown bone but the contour both before and behind this angle, indicates that it was inturned and blunt. The pterionic angle in the Swanscombe parietal did not form part of a sharp Sylvian falx as in modern skulls. The frontal is missing yet one may infer that its Sylvian margin was blunt. In Text-fig. 5 I have traced the frontal outline of the Piltdown, for, seeing the number of resemblances I have cited between the two, it is probable that the Swanscombe frontal was moulded on Piltdown lines—not Neanderthal—for in Neanderthal skulls the great wing of the sphenoid has a wide parietal contact at the pterion; in the Swanscombe skull this was not the case. We must also keep in mind that the Swanscombe parietal and occipital bones are modelled much more as in modern neanthropic skulls than is the case in the corresponding Piltdown bones.

Below the Sylvian ridge (Text-fig. 5*c*) is the impress of the first temporal gyrus, narrower than in Piltdown. Below the first temporal gyrus, the impress of a wide and highly developed second temporal, more extensive than in the

Piltdown parietal. It must be remembered that, when the Piltdown and Swanscombe outlines are superimposed, so that their parietal arcs correspond, the hinder part of the squamous margin of Swanscombe descends 5–6 mm. lower than that of Piltdown (Text-fig. 25, Part II). In other words, the temporal of Piltdown formed a relatively larger area of the lateral wall of the cranial cavity than it did in Swanscombe.

Up to this point, I have been comparing the anatomical points wherein Piltdown and Swanscombe agree. We have been considering those connected with the Sylvian region. To complete this analysis it will be convenient to consider here features of the Sylvian region which become manifest on the endocranial



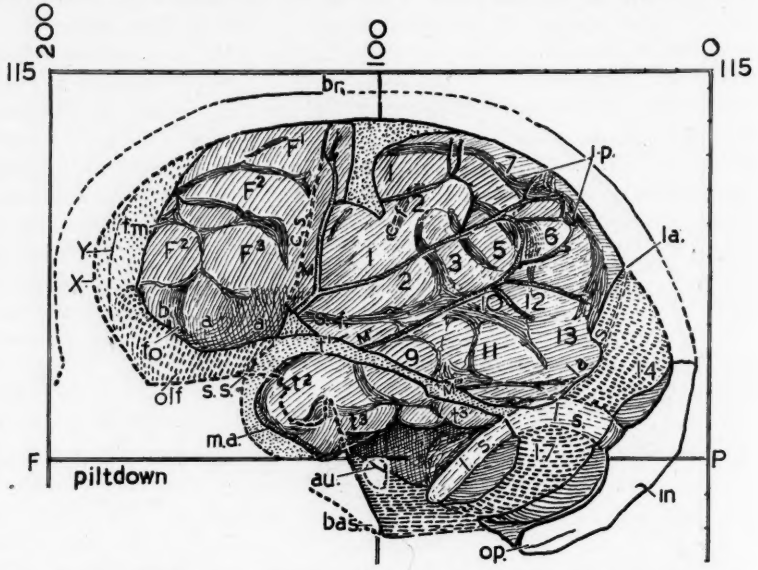
Text-fig. 5. The right aspect (interior) of the Swanscombe skull (shaded), on which has been superimposed an outline of the Piltdown skull (broken line). The lettering as in Text-fig. 4, with the following additions: *bo*, basi-occipital; *B*, dovetailed process, seen on its inner aspect; *x*, asterionic process of occipital.

cast and we may, at the same time, consider the anatomy of the lateral aspect of the brain, so far as this can be ascertained.

First let us look at the conformation of the third frontal convolution (F^3), which is more anthropoid than in any known fossil man. The anterior part, save a fragment which is missing (Text-fig. 6, *b*), and posterior part (*a*, *a'*) are preserved; the sulcus between these parts indicates the fronto-orbital fissure of the anthropoid brain (Keith, 1927). The area indicated by *a'* is the orbital and triangular opercula, but here, instead of rising into a bulging convexity as in neanthropic casts (Pl. I), the area is cylindrical and low as in anthropoids. In the anthropoid brain, the area marked *a*, *a'* forms a short

compact cylinder, whereas in the Piltdown cast it is greatly elongated. Just behind *a'*, where a break occurs, must have been the junction of the stem and posterior limb of the Sylvian (lateral) fissure (Text-fig. 6, *S.f*). It is probable that in Text-fig. 6 I have added too much to the pole of the temporal lobe, giving the stem (*ss*) a too oblique direction.

As to a description of the convolutionary pattern of the frontal lobe I would say this: we shall make no progress until we abandon the conventional nomenclature which has been too long in use. No one ever saw, in the brain of man or ape, a frontal lobe divided into upper, middle and lower tiers or

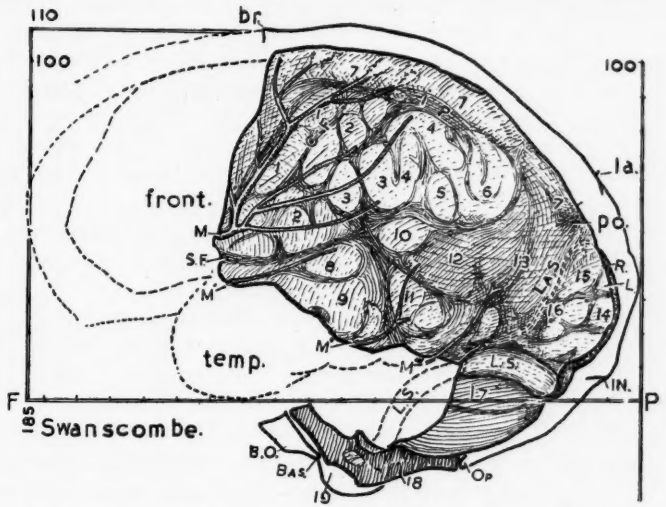


Text-fig. 6. Left lateral aspect of Piltdown endocranial cast, with outline of skull, set upon the Frankfort plane (F.P.). The missing areas are indicated by rough stippling. The position of bregma (*br*) lambda (*la*), inion (*in*), opisthion (*op*), basion, (*bas*) and external meatus (*au*) are indicated. For explanation of other lettering and numbering see text.

convolutions by two sulci running in an approximately parallel direction. The parts which I assign to the third frontal convolution in Fig. 6 are marked F^3, *b*, *a*, *a'*. The sulcus which separates F^2 and F^1 (Text-fig. 6) I believe to represent the accurate fissure of anthropoid apes; forwards, it is joined by a strong branch from the inferior frontal sulcus (above F^3), this branch dividing F^2 into two parts (F^2 F^2). This branch was apparently joined by the fronto-marginal sulcus (Text-fig. 6, *fm*). Although the convolutions are large yet the pattern is extremely simple and I think primitive.

We have only the parieto-occipital part of the Swanscombe endocranial cast: Is it possible to assess approximately the size of the missing temporal and

frontal parts? I think this is possible. In Text-fig. 7, I give a drawing of the left aspect of the cast and have orientated it on the same plane as the skull (Text-fig. 1). The cast, as seen in true profile, measures 122 mm. from occipital pole to the lower part of coronal suture. In human endocranial casts the frontal contributes 25–30 % of the total length; in anthropoid casts the frontal proportion is as large or larger. If we take 27 % as a probable estimate for the missing part of the Swanscombe cast, then the original length would have been 122 + 47 = 169 mm. Or if we suppose that the brain formed 90 % of the skull length then, according to our estimate, the original endocranial length was 168·5 mm. The frontal lobe in Mr Marston's reconstruction measures only



Text-fig. 7. Left lateral aspect of Swanscombe endocranial cast; without outline of parieta and occipital bones. The outlines of the frontal and temporal lobes were obtained by superimposing this drawing with that shown in Text-fig. 6, so that the Sylvian (lateral) fissures were in agreement. The specimen was orientated on the plane used for the skull (Text-fig. 1) which is an approach to the Frankfort plane. The lettering and numbering correspond to those used in Text-fig. 6. Further explanation in the text.

19 % of the total length. In the Piltdown endocranial reconstruction (Text-fig. 6) the total length is 177 mm.—the frontal segment making up 60 mm. of this amount—this being 34 % of the whole. This proportion is certainly too high and is due to a too liberal addition to the upper frontal. The true anterior limit is indicated in Text-fig. 6; it should be *Y*, not *X*, the total length being about 172 mm., the frontal forming a little over 30 % of the total length. In the endocranium of a chimpanzee the frontal component is 35·4 %; in an orang, 28·5 %. Thus, as regards total length of brain there is every reason to suppose that Piltdown and Swanscombe fell well within the dimensions which prevail amongst modern races of mankind. As regards height of brain it is

otherwise; both Piltdown and Swanscombe are low. It is a fortunate circumstance that in both man and anthropoid, the Frankfort plane corresponds approximately to the level of the floor of the middle fossa and therefore marks the lower limit of the temporal lobe. The height of the endocranium may therefore be measured from this plane as a base. The highest point of the Swanscombe endocranium rises 105 mm. and the Piltdown 102 mm. above this plane, as shown in the reconstructions depicted in Text-figs. 6, 7. The Sylvian fissure (*S.f*) is more horizontal in position in Piltdown than in Swanscombe. In both the impression which represents this fissure on the cast, is rather wider than is usual in those from modern skulls but anatomists will find no indication of the abnormal arrangement of parts described by Mr Marston. Nor is there any reason to suppose that the temporal lobe was relatively small in either Piltdown or Swanscombe. As reconstructed in Text-figs. 6, 7, the anterior part of *S.f* is situated 42 mm. above F.P. in both reconstructions. Relatively to the total height the temporal lobe is not defective in either of these two fossil brains. I have also applied to these reconstructions the system of measurements and indices invented by Dr Ariens Kappers (1932) but have found no measurement or index which is distinctive of either fossil.

It is not my intention to give an analysis of the cerebral features represented on the endocranial casts. As regards the region of the central fissure and the gyri in front of and behind it (Text-figs. 6, 7, *1*, *1*, *2*, *2*) and the region of the parietal above the interparietal fissure (*ip*) there are no clear indications of any departure from the normal, but it is otherwise in the inferior parietal lobule. The convolutionary eminences are more numerous and elaborate in this region of Swanscombe and rise sharply above the surrounding levels. In Swanscombe (Text-fig. 7) I identify *3*, *4*, *4*, as supra marginal, *5* as angular and *6* as the post-parietal gyri. These three areas are indicated in Piltdown (Text-fig. 6) by *3*, *5*, *6*; they are simpler and rise less prominently than in Swanscombe. In both, the inferior parietal seems to be evolving towards the modern state but I regard the condition in Swanscombe as the more advanced.

As regards the temporal impressions, we have only those which lie beneath the parietal bone in Swanscombe, whereas we have also those which lie within the temporal in Piltdown. The temporal and temporo-occipital regions, under the parietal bone, are more extensive in Swanscombe than in Piltdown. In Swanscombe, the eminences of the first and second temporal gyri, immediately below the auditory centre, marked *8*, *9*, in Text-fig. 7, are more extensive than in Piltdown. The late Sir Grafton Elliot Smith attached importance to these areas, as indicative of a power of speech. Behind these in both Swanscombe and Piltdown are two other eminences marked *10*, *11* (Text-figs. 6, 7) but in Swanscombe these eminences are more widely separated. Behind these in Swanscombe comes a peculiar depressed and extensive temporo-occipital area (Text-fig. 7, *12*), the significance of which I do not know; it is of less extent and is less depressed in Piltdown (Text-fig. 6, *12*). Behind the depressed triangular area, descends a sulcus, which seems to be connected with the hinder end of the

intra-parietal fissure. Still further back in Swanscombe comes the line of the lambdoid suture (*LAS*). Behind this suture in Swanscombe is the elaborately convoluted occipital cortex, projecting as a cap. In the reconstruction of Piltdown, the occipital cortex is shown in the same cap-like or bun-like form. In the casts of both Swanscombe and Piltdown the indications on the temporal and temporo-occipital regions are of a simplicity beyond what is usually met with on the endocrania taken from skulls of modern races.

As viewed in profile the cerebellum of Piltdown is seen to bulge downward and backward more than in Swanscombe; in other words, the cerebellum of Swanscombe is flatter and pressed upward and forward more than in Piltdown. The condition in Swanscombe approximates more to the Neanderthal type than is the case in Piltdown.

When we view the endocranial casts from behind, we shall see that Piltdown and Swanscombe are alike in this: the temporal gyri project laterally more than any of the parietal gyri (Text-figs. 10, 15), the greatest diameter of the brain lies low down in the temporal region. This, too, is a primitive or anthropoid character.

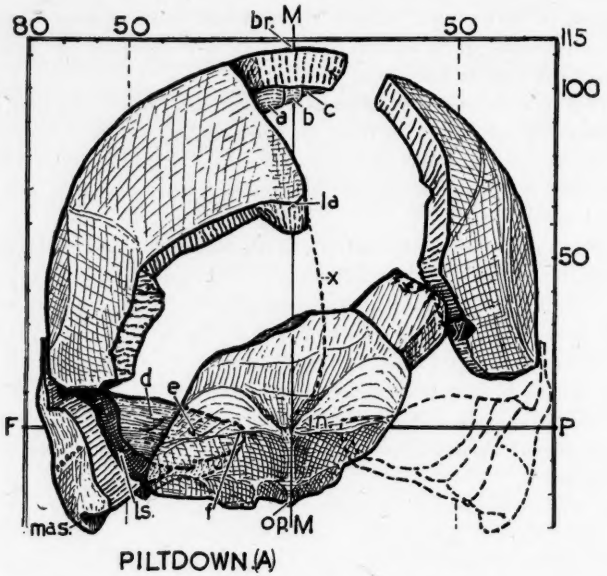
In Pl. I are reproduced illustrations of the Piltdown endocranial cast taken from photographs. In fig. 1 the left aspect of the fronto-parietal region of the cast is shown in profile; below it, to bring out the contrast between it and a cast from a modern skull, is shown, in fig. 2, the endocranial cast from the skull of a negro child aged 5 years. The difference in form and size of the third frontal gyrus (*a*) is very evident; so is the contrast in the width of the post-convolutionary depression (*b*). In both the anterior (post-coronal) branch of the middle meningeal vessels (*c*) and the linear depression of the lateral fissure (*d*) are indicated.

In fig. 3 is reproduced a photographic view of the vertex of the Piltdown cast. It is important, particularly when attempting a reconstruction of the entire skull, to note three markings in the upper region of the frontal lobe, the area lying under the bregmatic fragment. These are (*a*) the impress caused by the convexity of the mesial margin of the left frontal lobe; (*b*) a depressed linear area indicating the longitudinal sinus; (*c*) an area, partly damaged, caused by the mesial margin of the right frontal lobe. The sinus depression lies directly under the anterior end of the sagittal suture, which can be identified on the outer aspect of the bregmatic region of the skull. This should lie in the mid-sagittal axis of our reconstruction. At the hinder end of the cast, in the region of the lambda, the margin of the cast is sharply depressed or turned inwards. The depression of the margin I suppose to indicate the presence of an internal sagittal crest, produced at the hinder end of the sagittal suture. Such a crest is not uncommon in human skulls; an example is illustrated in Text-fig. 3B, *cr* (p. 162).

My main object, so far, has been to indicate the anatomical resemblances of the Swanscombe skull to that of Piltdown. I now turn to a consideration of

the reconstruction of the Piltdown skull in order to ascertain what measure of agreement now prevails as to its original size and shape, and how far features of the Swanscombe specimen help us to resolve our former difficulties and differences.

In Text-fig. 8 the Piltdown fragments are set up in the Frankfort plane, with anatomical points placed as in human skulls in which right and left sides are approximately symmetrical. The frontal fragment is seen to pass to the right of the middle line (*MM*) to an extent of 15 mm. This brings the anterior end



Text-fig. 8. Occipital view of Piltdown skull as seen with skull placed in the Frankfort plane. F.P. Frankfort plane; *MM*, median sagittal plane; *a*, impression of mesial margin of left hemisphere; *c*, of right ditto; *b*, raised impress of longitudinal sinus; *br*, bregma; *la*, lambda; *d*, hinder aspect of petrous; *e*, internal auditory meatus (stippled); *f*, apex of petrous; *ls*, lateral sinus; *op*, opisthion; *in*, inion. The upper horizontal is placed 115 mm. above the Frankfort plane; the lateral verticals are 80 mm. from the mid-line.

of the sagittal suture (*br*), as to the identification of which Elliot Smith and I have always agreed and which is also accepted by Friederich and Weinert, into the middle line of the skull. The impress for the longitudinal sinus (*b*) is also brought into the middle line; this linear area is flanked right and left by the impression of the mesial margins of the right and left cerebral hemisphere (*a*, *c*). The bregma, which is on the highest part of the vault, rises 113 mm. above the Frankfort plane. How far this differs from my original reconstruction and from those of later reconstructions will be seen at a glance in Text-fig. 10.

As to the relationship of the posterior-superior angle of the left parietal to

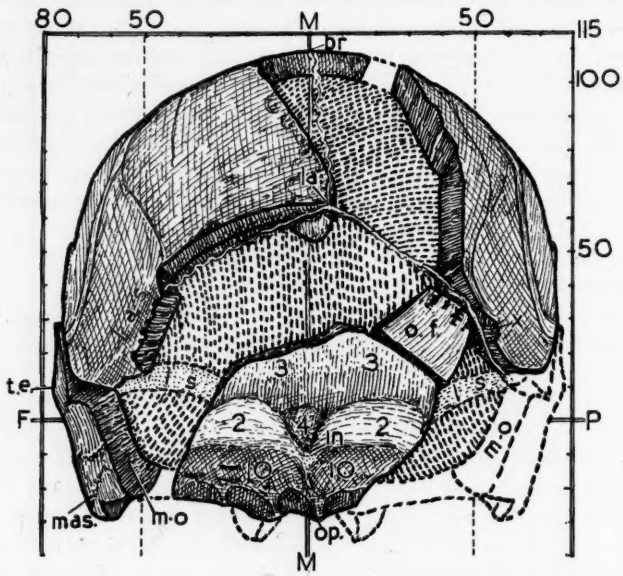
the middle line there is still a wide difference of opinion (see Text-fig. 10, p. 173). I still hold that the sharp ridge on the inner margin of the posterior superior angle of the parietal lay in the sulcus between the right and left hemispheres of the brain. A similar ridge or crest (*cr*) was present in the Egyptian skull drawn in Text-fig. 3 B (p. 162). In the Piltdown skull the longitudinal sinus was buried between the hemispheres in the posterior part of its course—under the parietals and under the squama of the occipital—so far as this part of the occipital is preserved. Nor is there any doubt that the sulcus between the right and left occipital lobes diverged to the right (Text-fig. 8, *x*). The left occipital lobe, in its lower part lay 10 mm. to the right of the true mid-line which is indicated on the outer surface of the nuchal area of the occipital bone. It is probable that this asymmetry was present not only in the occipital but also in the parietal lobes anterior to the lambda. The point marked as lambda (*la*) in Text-fig. 8 must be 2 or 3 mm. to the left of the true lambda, an identification in harmony with that of Friederichs'. In any attempt at reconstruction the anatomist will seek to keep the points (*br*, *la*) indicated in Text-fig. 8 in the middle line of the vault. The mid-line of the occiput is determined by the crest which passes from opisthion (*op*) to inion (*in*).

As regards the mid-line of the base of the skull we have guidance from the apex of the petrous bone. In Text-fig. 8, I have drawn the posterior aspect of the petrous, seen through the occipital fragment; the apex of the petrous is made to reach within 15 mm. of the mid-line. In my original reconstruction (1913) I strove to bring it within 13 mm., for I supposed the basilar process between the apices of the petrous was not likely to be more than 26 mm. in width. In a *Sinanthropus* skull the basilar process is 28 mm. wide. I found it impossible to get the several parts to fit unless I gave it the width shown in Text-fig. 8. It is probable that a fragment is missing from the apex. No reconstruction can be accepted for serious consideration unless the position of the petrous is shown.

The apex of the petrous has another important relationship to the base of the skull. The ridge which separates the two endocranial surfaces of the petrous, ends in its mesial direction just above the suture which separates basi-occipital from basi-sphenoid. We need a name to indicate the mesial end of this ridge and from lack of a better I shall use the term *apicon* for this point. In Text-fig. 8 the apicon is represented as 4 mm. above the F.P.; the lateral end of the ridge is 11 mm. above that plane; the ridge, as in anthropoids, slopes downwards and inwards in this case at an angle of 14°; it also slopes inwards and forwards making an angle of 44° with the mid-line of the base. Friederichs claims that the upper border should not slope downwards, but be horizontal as in many skulls of modern races. Weinert on the other hand represents the apicon as being 5 mm. below F.P., a position found neither in human nor simian skulls.

Now, to retain the apicon within 15 mm. of the mid-line we have to keep the cranial width, both behind and in front, to the narrowest possible limits. As in

1913, I found, if the apicon was to be retained in position, the forehead must be modelled on narrow lines; the minimal frontal width in my present, as in my first, reconstruction is 103 mm. and the supra-orbital width (measured between the most laterally placed points of the fronto-malar suture) 108 mm. In his reconstruction of 1927 Elliot Smith gave this narrow width to the frontal; Friederichs gives the minimal frontal width as 99 mm.; Smith Woodward and Weinert give the lower frontal a great width (see Text-fig. 29, Part II).



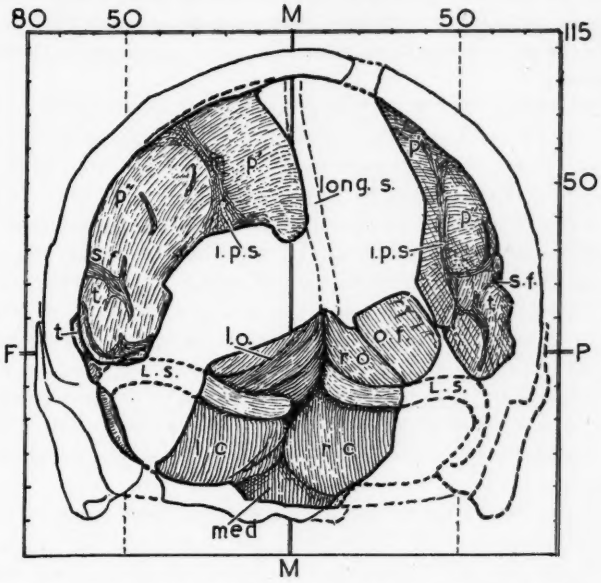
Text-fig. 9. Occipital view of my definitive reconstruction of the Piltdown skull. F.P. Frankfort plane; *MM*, mid-sagittal plane; *br*, bregma; *la*, lambda; *in*, inion; *op*, opisthion, *o.f*, occipital fragment; *x*, perforation; *las*, lambdoid suture; *l.s*, lateral sinus; *te*, temporal (supramastoid) eminence; *mas*, mastoid process.

The position of parts shown in Text-fig. 8 represents the highest region of the vault as rising 113 mm. above the F.P., 4 mm. less than in my reconstruction of 1913. This is due to my recognition that less was missing from the regions of the asterion and pterion than I had originally supposed; in my present reconstruction the temporal has been moved forwards on the parietal to an extent of 4 mm. and the mastoid and squamous parts have been more closely fitted to the left parietal.

When I sought to reconstruct on the lines shown in Text-fig. 8, I came against two difficulties; the bregmatic fragment and its endocranial cast of the left side, projected upwards so much that they would not run into the contour of the adjacent parts of the right parietal—particularly of its endocranial surface. It was only when I depressed the bregmatic fragment to an extent of 3 mm.,

and thus pressed the anatomical mid-line towards the right, that I got an approximately harmonious arrangement of parts. And when the bregmatic fragment is so depressed, the region of the lambda is also necessarily depressed and passes farther to the right, giving the position of parts depicted in Text-fig. 9.

In Text-fig. 9 the bregmatic fragment has been depressed so that the auricular height in my definitive reconstruction is 110 mm. The greatest



Text-fig. 10. Occipital view of the Piltdown endocranial cast—taken from the reconstruction shown in Text-fig. 9, and poised similarly. The outline of the skull is also depicted. The position of the longitudinal sinus (*long. s*) is indicated; so are the right and left lateral sinuses (*l.s*); *ips*, intraparietal sulcus; *sf*, Sylvian fissure; *lo*, left occipital pole; *ro*, right occipital pole; *P'* upper, *P''*, lower parietal lobule; *t*, *t*, temporal lobe; *lc*, left hemisphere of cerebellum; *rc*, right hemisphere; *med*, medulla oblongata.

parietal width 150 mm.; greatest temporal width—taken between the supramastoid eminences (Text-fig. 9, *te*)—154 mm. The bregma is now 2 mm., the lambda 6 mm. to the right of the mid-line. I have to presume there is not only asymmetry of the brain but also of the skull. In Sir A. Smith Woodward's original reconstruction the point I have marked *la* (Text-fig. 9) passes 16 mm. to the right of the mid-line; in Elliot Smith's (1927) 10 mm.—the degree of asymmetry being greater in their reconstructions than in mine.

In Text-fig. 10 the bones have been removed from the view of the skull given in Text-fig. 9, and the brain, as represented by the endocranial cast exposed. The left occipital lobe is seen to deviate to the right of the mid-line

to an extent of 10 mm.; on the other hand the right half of the cerebellum crosses to the left of the mid-line to an extent of 3 mm. The longitudinal sinus must have diverged to the right before reaching the region of the obelion. It was submerged and did not appear on the surface until it passed into the right lateral sinus. The lateral sinuses are represented as reaching the same level as they approach the asterion; the medulla oblongata (Text-fig. 10, *med*) and foramen magnum are presumed to have had their normal symmetry in the mid-line of the base. It will be noted that the left occipital lobe of the cerebrum and the right half of the cerebellum make a wide contact. There can be no doubt as to the great degree of asymmetry in the arrangement of the structures just mentioned.

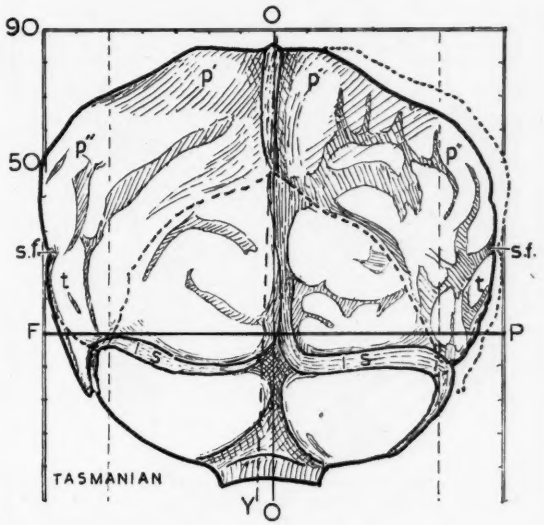
It is also certain that the degree of asymmetry in the parietal lobes, was equally great. The position of the Sylvian fissure (Text-fig. 10, *S.f*) is indicated on each side; so are the intraparietal sulci (*ips*). Now, in the left hemisphere the intraparietal sulcus approaches within 22 mm. of the mid-line; on the right hemisphere it is 45 mm. distant. In no reconstruction can the sulcus of the right side be made approximately symmetrical with that of the left side. Besides, on both right and left, the inferior parietal lobule is preserved (Text-fig. 10, *P''*) and that on the right is manifestly less in extent and in prominence on the right side than on the left. We have here evidence of a cerebral asymmetry beyond anything known in the brain of any other race. It is this high degree of asymmetry that has given rise to much of the discrepancy of our reconstructions. We had presumed that asymmetry was a mark of specialization and of modernity; the Piltdown discovery gives us convincing evidence to the opposite.

It so happened that amongst the endocranial casts I had available for comparison was one taken from the skull of a Tasmanian. It was poised as in Text-fig. 10 and drawn. The drawing is shown in Text-fig. 11. The longitudinal sinus departs a little to the left as it approaches the lambda, where it ceases to leave an impress. It reappears between the occipital lobes and ends in the right sinus. On the left, the lateral sinus almost reaches the Frankfort plane; on the right it falls 5 mm. short of that plane. The left occipital lobe crosses to the right of the mid-line to a distance of only 3 mm., yet the left lobe is manifestly the larger and has pushed the lambdoid suture far out to the left. The right parietal lobe, particularly the inferior lobule, is less prominent and less extensive than on the left—the difference being indicated by the stippled outline shown on the right side. As in the Piltdown cast, the intraparietal sulcus on the right side is much farther from the mid-line than on the right. Yet, compared with the Piltdown cast, we note the great parietal development in the Tasmanian which quite overshadows the temporal lobe in its lateral prominence: In the Piltdown as in the Swanscombe cast, the temporal lobes are the more prominent laterally, as seen in an occipital view (Text-figs. 10, 15).

If we orientate the cast so as to bring both lateral sinuses into the Frankfort

plane, as would be the case if we adopted the broken line *Y* as our mid-plane, then the parietal asymmetry is lessened, but the occipital assymmetry is increased, and the medulla and foramen magnum must be moved towards the right of the mid-line of the base. However we orientate the cast we have a considerable degree of asymmetry, not so great as in the Piltdown skull, yet enough to make a reconstruction from mere fragments a task of difficulty.

A further study of the asymmetry of the Piltdown endocranial cast is presented in Text-fig. 12. It will be seen that the groove for the longitudinal sulcus (Text-fig. 12, *b*) lies to the right of the middle line; so does the sulcus for

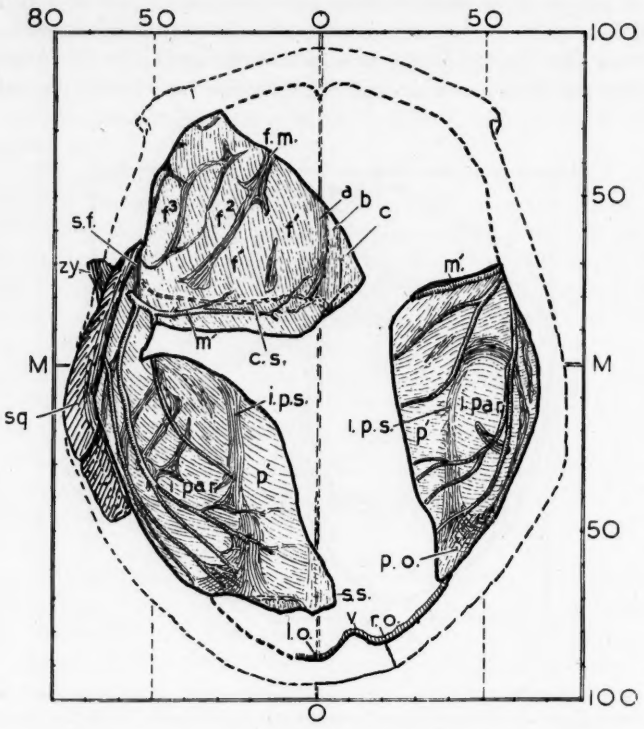


Text-fig. 11. Occipital view of an endocranial cast taken from the skull of a Tasmanian. It is poised as in Text-fig. 10. *O, O,* median sagittal plane. F.P. Frankfort plane. If the broken line ending in *Y* is taken as mid-plane, then the nature of the asymmetry is altered. The stippled line on the right, indicates the extent to which the left parietal area exceeds the right. Other letters have the same significance as in Text-fig. 10.

the sagittal crest in the region of the lamba (Text-fig. 12, *ss*). The marked asymmetry in the position of the right and left intraparietal sulcus (*ips*) is also made evident; the right inferior parietal lobule is manifestly less developed on the right than on the left; the left occipital lobe (Text-fig. 12, *lo*) projects 5 mm. farther back than the right lobe and extends 10 mm. to the right of the mid-line.

Two other points may be noted in Text-fig. 12. The lateral margin of the inferior frontal convolution on the left extends 52 mm. from the mid-sagittal plane, giving a frontal width of 104 mm. The most lateral point of the temporal

lobe, which lies immediately behind the mid-auricular plane (Text-fig. 12, *MM*), is 67 mm. from the mid-line, giving a maximum width to the brain of 134 mm. As in modern skulls, the greatest width is far forwards, as contrasted with the posterior position of the greatest width on endocrania of Neanderthal



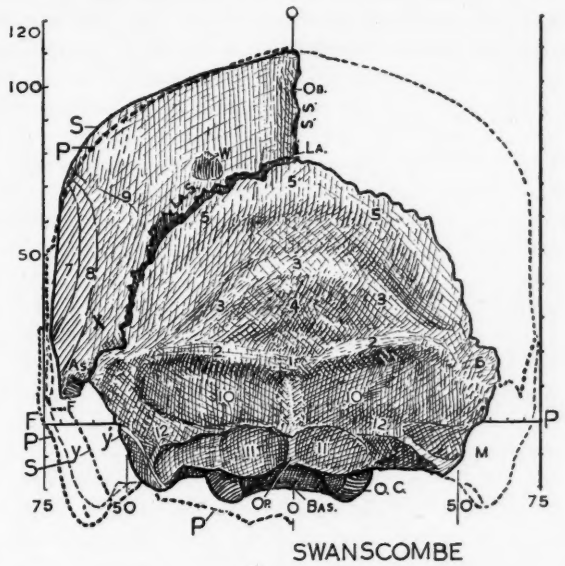
Text-fig. 12. Vertex view of Piltdown endocranial cast, at right angles to F.P. The outline of the skull is shown, the left temporal bone (*sq*) being shaded. *O, O*, sagittal median plane; *MM*, mid-auricular plane. *a*, mesial margin of left frontal lobe; *b*, impress of sinus; *c*, the mesial margin of right frontal lobe. F^1F^1, upper frontal convolution; F^2, middle, F^3, lower frontal; *fm*, arcuate running into fronto-marginal sulcus; *cs*, line of coronal suture; *m′*, post-coronal branch of middle meningeal vessels; P^1, upper parietal lobule; *ipar*, inferior parietal lobule; *ips*, interparietal sulcus; *po*, parieto-occipital region of right side; *ss*, sulcus for sagittal crest; *lo*, left occipital lobe; *ro*, right occipital lobe; *v*, interpolar depression; *S.f*, Sylvian fissure; *zy*, zygoma.

man. The narrow pointed form of the occipital end will also be noted—in contrast to the blunted form in Neanderthal skulls.

The preceding pages have been devoted to a demonstration of the degree of cerebral asymmetry in Piltdown man. I now return to a consideration of the

Swanscombe skull and its comparison with Piltdown as reconstructed in Text-fig. 9.

As drawn in Text-fig. 13, the Swanscombe skull is given a parietal width of 146 mm.; on the actual cast, this measurement was 144 mm. It is thus less in width than the Piltdown specimen (150 mm.), but this difference is due to the



Text-fig. 13. Occipital view of the Swanscombe skull, orientated on the plane shown in Text-fig. 1. F.P. Frankfort plane; *O,O*, sagittal median plane. On the right side a stippled line represents an outline copied from that of the left side. On the left side, the inferred form of the Swanscombe mastoid (*S*) is represented by a broken line. The heavily stippled line (*P*) on the left side represents the left side of the Piltdown skull as reconstructed in Text-fig. 9 (p. 172). *i*, inion; *2, 2*, on the nuchal wave or ridge of bone, the numerals being placed above the linea nuchae superior and below the linea suprema; *3, 3,3*, supranuchal ridge or wave; *4*, supranuchal area; *5, 5*, infralambdoid ridge; *6*, asterionic angle; *7, 8*, temporal lines; *9*, supreme temporal line; *10, 10*, area for complexus insertion; *11*, insertion of rect. cap. post. minor; *12*, insertion of superior oblique. *oc*, occipital condyle; *bas*, basion; *PPP*, stippled outline of Piltdown—from Text-fig. 9. *SS*, Swanscombe. *As*, asterion; *x*, supra-asterionic field; *y*, occipital border of mastoid (Piltdown); *y'*, mastoid border of occipital (Swanscombe); *M*, mastoid. The basion (*bas*) in this drawing is shown at a lower level than the opisthion, having been made from my first orientation—with the bregma 4 mm. lower than the verticon.

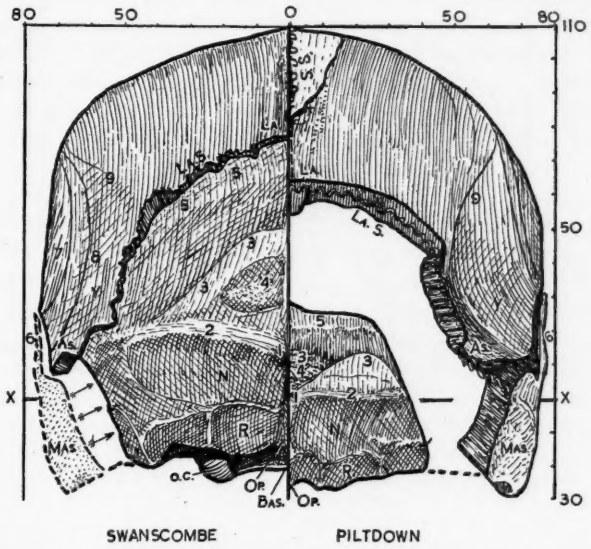
greater thickness of the Piltdown parietals, for the greatest width of their endocranial casts is almost identical. The total height of the skull, from the plane of the foramen magnum to verticon, is 135 mm.; this measurement is greater in Piltdown, being 140 mm. The excess is due in part to the greater thickness of the Piltdown bones, but it is also due, as we shall see later, to a greater fullness and depth in the cerebellar region of the Piltdown occiput.

The most noteworthy feature of the Swanscombe occipital bone is its greatest width. This is due to the exceptional development of its asterionic angles: The distance, measured between the most lateral points by calipers, amounts to 122 mm.; the arch between these points, measured by tape, along the curved lines, is 168 mm.; the subtense of the arch, 13·5 mm. In the reconstruction in Piltdown, shown in Text-fig. 9, the corresponding measurements are: 130 mm., 165 mm.; 12 mm. All who have attempted the reconstruction of the Piltdown skull, with the exception of Dr Friederichs, have recognized that it must have had a great occipital width. Elliot Smith estimated the greatest width to be 126 mm., slightly more than in Swanscombe; Smith Woodward 128 mm., whereas I make it even greater, 130 mm. In Text-fig. 13, there are shown on the left side, a stippled outline of the mastoid process in both Piltdown and Swanscombe. In Piltdown the lateral outline of the mastoid at its base is only 3 mm. farther out than the outline I have given of the mastoid of Swanscombe. But it will be observed that the margin of the mastoid which articulated with the occipital (Text-fig. 13, *y*) is 10 mm. farther out than the corresponding margin of Swanscombe (Text-fig. 13, *y*). I attempted in every way to bring the Piltdown mastoid inwards to make it meet the Swanscombe occipital but failed to obtain a contact, except by producing deformity elsewhere. The mastoid part of the Piltdown temporal at its occipital aspect, is bent inwards to a less extent than in other skulls.

Very peculiar, too, are the markings on the outer aspect of the Swanscombe occipital. These are given a semi-diagrammatic expression in Text-fig. 13. The region of the inion (*1*) is scarcely raised into a protuberance. On each side are *2*, *2*, a ridge or wave of bone, bounded below by the linea nuchae superior, above by the linea nuchae suprema. Half-way up the squama is a crescentic ridge or wave (*3*, *3*, *3*); between *2*, *2* below and *3*, *3*, *3* above, is a rough depressed area (*4*) which I shall speak of as the supranuchal area. Still higher up, bordering the lambdoid margin, is another crescentic elevation, the infralamboid crescent (Text-fig. 13).

The markings on the Piltdown occipital are also peculiar (see Text-fig. 9, p. 172). The ridges *2*, *2* are much wider, the linea suprema being higher up and better defined than in Swanscombe. Then, just below the fractured margin of the Piltdown occipital, is part of a ridge, which I believe to represent *3*, *3* in the Swanscombe bone; *2*, *2* are separated from *3*, *3*, *3* by a depressed supranuchal area; that which is marked *4* (Text-fig. 9) appears to correspond to a similar area above the inion in Swanscombe. Then in Piltdown the parts of the occipital which I believe carried the infralambdoid crescent are missing; there is a trace of such an elevation in the occipital fragment of Piltdown (Text-fig. 9, *of*). The outer markings of the occipital in Swanscombe differ very considerably from those of Piltdown, so far as the latter are known to us, but it does seem possible that those of Swanscombe may be derived from those of Piltdown. In these markings Piltdown and Swanscombe bear a closer resemblance to those of modern crania than to those which particularize Neanderthal skulls.

In Text-fig. 14 I give an alternative interpretation of the external markings of the Piltdown and Swanscombe occipital bones. On the left side is shown the left half of Swanscombe; on the right, the left half of Piltdown reversed. The inion (*1*) in Swanscombe is situated 14 mm. higher than in Piltdown. Here I have attempted, unsuccessfully I think, to correlate *3*, *3*, in Swanscombe, with the linea suprema in Piltdown, and the infralambdoid in Swanscombe (*5*, *5*) with the upper ridge on the Piltdown fragment (*5*); such an interpretation implies that the area marked *4* in Piltdown has expanded into the large supranuchal area of Swanscombe. It will be observed that the lambda (*la*) of Swanscombe is situated 12 mm. above the corresponding point of Piltdown. In



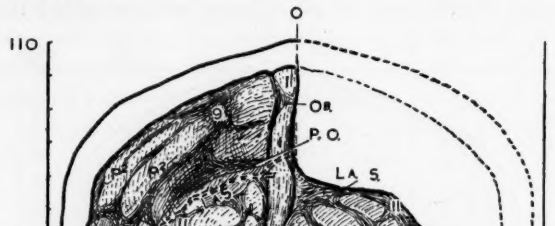
Text-fig. 14. The left half of the occipital aspect of the Swanscombe skull compared with the left half of the Piltdown skull, the latter being reversed and placed on the right. *x*-*x*, Frankfort plane; *O*,*O*, vertical sagittal plane; further explanation in the text.

both the area for attachment of the complexus (*N*) is extensive, but more so in Swanscombe than in Piltdown. The area for the rect. cap. post. minor (*R*) is also larger in Swanscombe.

Text-fig. 14 also brings out the impossibility of articulating a mastoid process, shaped as in Piltdown, with an occipital shaped as in Swanscombe. Three arrows mark the gap which exists when the mastoid of Piltdown is transposed to the left side; even if pushed inwards until the squamous articulation is closed, a masto-occipital gap remains.

One other difference between Piltdown and Swanscombe is brought out in Text-fig. 14. As is well known, the area of the parietal (Text-fig. 14, *y*), which

lies between the temporal lines and the lambdoid suture, forms a peculiar field; I will speak of it as the supra-asterionic field. Along the lambdoid border of the parietal, from the asterion upwards, there is a raised ridge of bone—the pre-lambdoid ridge. It corresponds to the post-lambdoid ridge of the occipital —both ridges being vestiges of the components which go to make up the lambdoid crest in Anthropoid skulls. The peculiar supra-asterionic field of the Piltdown skull is due to the development of a pre-lambdoid ridge. This ridge is but slightly developed in the Swanscombe skull and hence the field is less clearly demarcated.



Text-fig. 15. Occipital view of the Swanscombe endocranial cast, poised as in the drawing of the skull (Text-fig. 13). *1, 1, 1,* superior longitudinal sinus; *2,* left lateral sinus; *4,* right; *3, 5,* the asterionic segments of left and right sinus; *6,* left, *7,* right cerebellum; *8* medulla; *9,* intra-parietal fissure; *10,* parieto-occipital triangular area; *11,* post-lambdoid depression on left, lunate sulcus on right; *Fs,* Sylvian fissure; *t, t′,* temporal convolutions; P^2, P^3, inferior parietal lobule (angular and post-parietal); *ob,* obelion; *po,* parieto-occipital fissure; *las,* position of lambdoid suture, *A, B,* on upper polar convolutions above the emerging retro-calcarine sulcus; *CD,* on the lateral convolutions of the striate operculum.

In the foregoing pages I have compared the markings of the Swanscombe occipital with those of Piltdown. In Text-fig. 15, is given a drawing of the occipital aspect of the Swanscombe endocranial cast, for comparison with the corresponding view of the Piltdown cast (Text-fig. 10, p. 173). The Swanscombe occiput is complete whereas that of Piltdown is fragmentary. The first point to impress one is that in both there is a high degree of asymmetry; that of Swanscombe is the mirror image of Piltdown. In Swanscombe it is the right occipital pole and the left half of the cerebellum that are specialized and come into contact. The occipital areas in Swanscombe are extensive and highly convoluted. As reconstructed in Text-fig. 10 the occipital areas of Piltdown

are about equal in extent to those of Swanscombe, but these areas in Swanscombe were richly convoluted, as much so as in any modern brain. The area on the right is more extensive than that of the left and is limited laterally by a sharply defined crescentic sulcus which I infer to represent the lunate sulcus and the margin of the striate cortex, which extends over the lateral aspect of the brain to an unusual extent.

It will be noted that the longitudinal sinus (Text-fig. 15) lies to the left of the mid-sagittal plane, the opposite of the case in Piltdown (Text-fig. 10). We have seen that the right parietal areas of Piltdown, are much more developed on the left than on the right. If the same correlationship holds for Swanscombe, then the right parietal areas under the missing right parietal of Swanscombe should prove to be more richly developed than on the left.

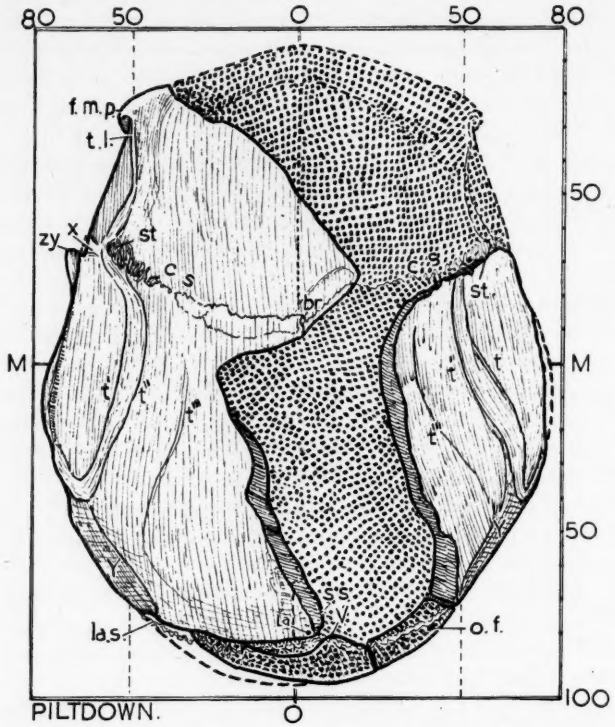
As regards other points brought out in Text-fig. 15, I would mention three: (1) the prominence of the temporal convolutions on the lateral aspect (Text-fig. 15, *t*, *t'*); (2) the depressed parieto-occipital region of the left side (Text-fig. 15, *10*); (3) the convolutionary pattern is more complex in Swanscombe than in Piltdown.

We now turn from an occipital view of the Piltdown and Swanscombe skulls to consider their vertical aspect. One view of this series has been dealt with already in connexion with asymmetry, namely the endocranial cast of Piltdown (Text-fig. 12). In Text-fig. 16 a view of the vertex of the skull of Piltdown is given; the widest part lies at and just behind the mid-auricular coronal plane, the squama of the temporal bone being visible laterally in this region. The anterior temporal lines (*tl*) are seen to fall just outside the 50 mm. latitude, giving a minimal frontal width of 103 mm.; the fronto-malar process (*fmp*) projects 4 mm. beyond the 50 mm. latitude, giving a supra-orbital width of 108 mm. The stephanion (*st*) is 60 mm. distant from the mid-line, giving a bi-stephanic diameter of 120 mm.; the maximal frontal width (at *x*) is 126 mm. The upper temporal lines will be seen in Text-fig. 16 (*t''*) to approach within 46 mm. of the mid-line of the skull. There is a supreme temporal line (*t'''*). In the hinder end are shown the thick internal median ridge of the occipital (*V*); *ss* the sagittal crest at the lambda (*la*); the right occipital fragment (*of*) and the position of the lambdoid suture (*las*). On each side is seen the supra-asterionic field (*y*), that of the right side being much flatter than on the left. The left occiput, completed by a stippled line, is seen to be more extensive and fuller than on the right.

Further, it will be noted that on the right the parietal fragment is given a more advanced position than on the left; the upper part of the right coronal suture is 4 mm. in front of the upper part of the left. It will be noted that on the bregmatic fragment the part of the coronal suture which is preserved there turns forward at a more acute angle than the corresponding part of the left (Text-fig. 16). I have noted, in skulls in which the left occiput projects backwards pronouncedly more than the right, that the right frontal eminence is placed anterior to the left and with this, the right coronal suture makes the

acute angle seen in the Piltdown skull. The break in the right parietal is only one or two millimetres behind the coronal suture and stephanion.

As drawn in Text-fig. 16, the total length is only 190 mm., being thus 4 mm. shorter than the corresponding profile view (Text-fig. 2). This is due to my having failed to orientate properly on the Frankfort plane and to have reduced the glabellar projection. As drawn the total length is 190 mm., the maximum

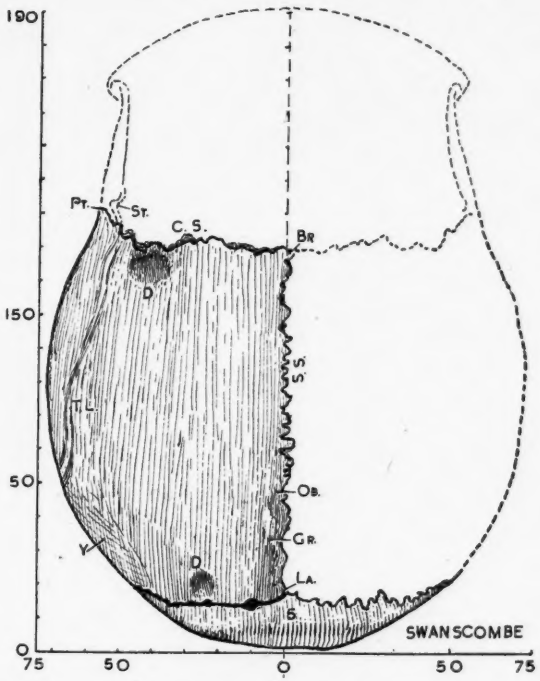


Text-fig. 16. Vertex of the reconstruction of the Piltdown skull, made at right angles to the views shown in Text-figs. 2, 9. The missing parts are stippled. *MM*, mid-auricular plane; *O,O*, mid-sagittal plane; *br*, bregma; *la*, lambda; *ss*, sagittal crest; *V*, internal occipital ridge; *cs*, coronal suture; *x*, lateral coronal ridge; *st*, stephanion; *zy*, zygoma; *fmp*, fronto-malar process; *tl*, anterior temporal lines; *t'*, *t''*, *t'''*, lower, upper and supreme temporal lines; *las*, lambdoid suture; *of*, occipital fragment; *yy*, supra-asterionic field.

width 154 mm.—the width representing 81 % of the length. Undoubtedly the Piltdown skull approached if it did not attain a place in the brachycephalic group.

In Text-fig. 17 a reconstruction is given of the vertex of the Swanscombe skull. The right parietal is indicated by duplicating the outline of the left. Seeing the many anatomical points in which the parietal and occipital of this

skull resembles those of the Piltdown skull, I feel justified in modelling the Swanscombe frontal on Piltdown lines, but in doing so I have carried the frontal outline 5 mm. too far forwards, giving a total length to Swanscombe of 190 mm., whereas our previous calculations gave its length as 185 mm. When the outlines of Piltdown, in Text-fig. 16, and of Swanscombe, in Text-fig. 17, are compared, there is seen to be a striking degree of resemblance between

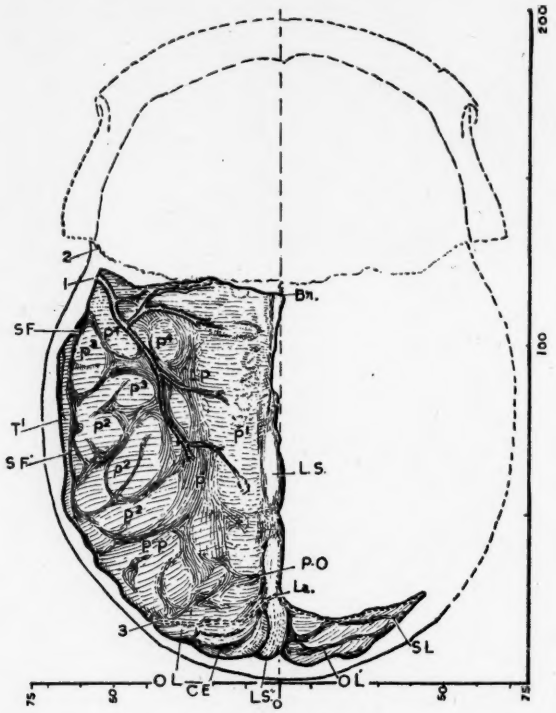


Text-fig. 17. Vertex view of the Swanscombe skull, drawn at right angles to the view given in Text-fig. 1. The parts found are shaded. The right parietal has been outlined, a mirror image of the left. The frontal outline is based on that of Piltdown shown in Text-fig. 16. *br*, bregma; *la*, lambda; *ob*, obelion; *gr*, post-obelial groove; *ss*, sagittal suture; *cs*, coronal suture; *st*, stephanion; *pt*, pterion; *tl*, temporal lines; *y*, supra-asterionic field; *DD*, depressions indicating healed injuries or ulcers. *5*, infralambdoid ridge of occipital.

them. The maximum width in Swanscombe, as shown in Text-fig. 17, is 145 mm., being 78·3 % of the estimated length. If we had the temporal bones of Swanscombe, we should have to add to the width, for the maximum width must have been in the temporal regions. The cephalic index of both skulls lay in the neighbourhood of 80.

The temporal lines, poorly marked in Swanscombe, lay farther apart in Swanscombe than in Piltdown, the left upper line approaching within 52 mm.

of the mid-line. In this Swanscombe is the more evolved form. On the other hand the maximum frontal width (bi-pterionic diameter) in Swanscombe was only 118 mm., against 126 mm. in Piltdown. Nevertheless, if allowance is made for the greater thickness of the bones in Piltdown, the difference in the intracranial diameter at this point is only 4 mm. The lambda is situated 15 mm. in front of the opisthocranion; the right occiput is fuller and wider than the left.



Text-fig. 18. Vertex view of the Swanscombe endocranial cast, drawn at right angles to the profile shown in Text-fig. 7. *br*, bregma; *la*, lambda; *ls*, longitudinal sinus; *1*, anterior branch of middle meningeal vessels; *2*, pterion; *3*, line of lambdoid suture; *OL*, *OL'*, occipital lobes. Further explanation in text.

The supra-asterionic field (Text-fig. 17, *y*) is merely a vestige of that seen in Piltdown (Text-fig. 16). In brief, the condition seen in the vertex of Swanscombe is highly evolved compared with that seen in Piltdown.

In Text-fig. 18 is presented a vertex view of the Swanscombe endocranium, set within the outline of its bones; the outline of the frontal represents a tracing taken from Piltdown. We note that the left occipital lobe (*OL*) is smaller and less projecting than the right (*OL'*) and that the longitudinal sinus lies to the

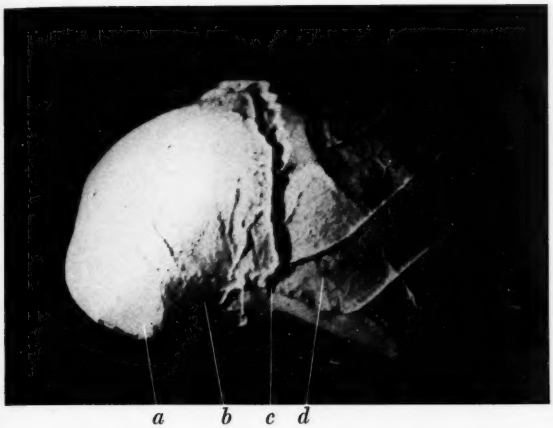


Fig. 1.

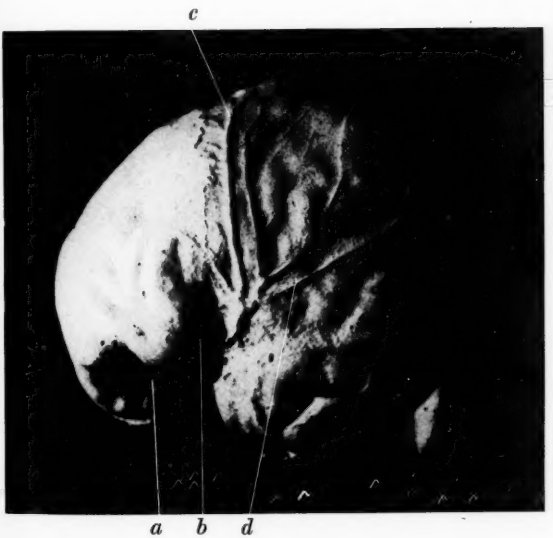


Fig. 2.

Fig. 3.

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left of the middle line, whereas in Piltdown, it is to the right (Text-fig. 12, p. 176). The sinus is not submerged as in Piltdown, but has made an impress on the skull for the greater part of its course. The intraparietal sulcus (*P*) approaches within 25 mm. of the mid-line, the same distance as in the left hemisphere of Piltdown (Text-fig. 12). The elevations P^4, P^4, I identify with the precentral gyrus, P^3, P^3, with the post-central. P^2, P^2, P^2, appear to represent the supra-marginal and angular gyri of the inferior parietal lobule; *PP*, the post-parietal gyrus. The Sylvian fissure (*SF*) is well marked, with the temporal gyri (t') lying lateral to it as in the Piltdown cast (Text-fig. 12).

A depressed area, anterior to the lambda, indicates the region of the parieto-occipital fissure. All of these structures are more clearly demarcated in Swanscombe than in Piltdown, indicating, as it seems to me, a more highly organized brain. Behind *PP* (Text-fig. 18) is the flat unexpanded parieto-occipital region. On the right side *SL* marks the sulcus lunatus and *CE*, on the left, the external calcarine fissure.

DESCRIPTION OF PLATE I

Fig. 1. Photograph of the left aspect of the Piltdown endocranial cast (fronto-parietal fragment). *a*, Third frontal convolution; *b*, post-frontal depression; *c*, middle meningeal artery; *d*, lateral fissure.

Fig. 2. Left aspect of endocranial cast from skull of a negro child, aged 5 years. Lettering as in Fig. 1.

Fig. 3. Photograph of vertex of the fronto-parietal part of the Piltdown endocranial cast, the light being thrown so as to bring out *a*, the impress of the mesial margin of left frontal lobe; *b*, the smoother and partially submerged area for the longitudinal sinus; *c* the imperfectly preserved impress for the mesial margin of right frontal lobe; *d*, the sharp depression caused by a sagittal ridge. (Photographs by F. Watson.)

(*To be continued*)

THE MORPHOLOGY OF THE SAPHENOUS OPENING

By C. R. SALSBURY

Department of Anatomy, Queen's University, Kingston, Canada

It has been suggested, in a recent article (Salsbury, 1937), that the femoral sheath is formed by a downward movement of the femoral vessels when the thighs are first extended from the intra-uterine position of extreme flexion. The evidence presented may be briefly summarized.

(1) Such an hypothesis explains satisfactorily the anatomical structure of the femoral sheath and throws some light on the etiology of femoral hernia.

(2) There is no femoral sheath in the foetus. To supplement our own dissections, we have the findings of Panton (1922).

(3) Dissection of the foetus had led to the observation that the deep circumflex iliac and inferior epigastric arteries were at a relatively higher level than in the adult. To check the accuracy of this general conclusion, comparative measurements were made in a series of foetal and adult bodies. The levels of origin of the vessels were measured with reference to a line from the pubic tubercle to the anterior superior iliac spine. A growth ratio was established by measuring the femurs, from the highest point of the greater trochanter to the most inferior part of the lateral condyle. The average level of origin in the foetus and the growth ratio were used for the calculation of the expected level of origin in the adult. It was found, however, that the actual adult levels were considerably inferior to those established by computation. The variation averaged 10·1 mm. for the deep circumflex iliac artery and 13·5 mm. for the inferior epigastric and was accepted as the amount of proven descent. It was pointed out, however, that the adult measurements were from vessels that had been partially exposed by students some weeks earlier and, since the femoral vessels were cut and might shrink upward, the average adult levels would be too high and the range of the downward movement too small. This suggestion was confirmed in a new series to be recorded below.

A later observation offers some support and appears worth recording. The fallacy of attempts to measure the length of the sheath was pointed out, but the size of the femoral canal may be of anatomical significance. In the absence of femoral hernia, or the fatty mass which appears to precede it, as suggested by Buckley (1924), one may offer the generalization that *the lower the origin of the deep circumflex iliac and inferior epigastric arteries, the greater the length of the femoral canal.* Statistical proof of this statement has not been attempted, but I believe that careful observation will convince anyone of its accuracy.

Since the day when, as a student, I first saw the saphenous opening, I have felt that its appearance could be due only to traction. This old idea acquired new interest with investigation of the formation of the femoral sheath. Is not the saphenous opening in the fascia lata (fossa ovalis) a direct result of the downward movement of the femoral vein? Considerable evidence can be offered to support the belief that such is, indeed, its origin and significance.

(1) The saphenous opening of the foetus is a simple structure. The saphenous vein passes postero-laterally through a hole in the fascia lata and the margins of this aperture have none of the tension so characteristic of the adult structure.

The importance of the remaining evidence will be more easily understood if we digress, at this point, to consider the probable changes attendant on extension.

The femoral triangle is described as extending from the medial border of the sartorius to the medial border of the adductor longus. The fascia lata is said to form the roof, thereby giving the impression that it extends from the lateral to the medial boundary. Such a conclusion is definitely incorrect. The fascial layer, stretching medially from the sartorius, fuses with the deeper fascia immediately medial to the femoral vein. The deep attachment is mainly to the fascia of the pectineus but, inferiorly, a small portion reaches the fascia of the adductor longus. The true arrangement is very obvious on dissection but, so far as I am aware, has not been fully described in any of the textbooks, although Cunningham's *Manual* (1935) illustrates it correctly.

Let us imagine the flexed foetal position, with the fascial attachments as described, and the saphenous vein passing through a simple aperture in the fascia lata, or between the edge of that fascia and the surface of the fascia of the pectineus. The aperture is merely large enough to admit the vessel, along with some superficial arteries and lymph vessels. The limb is extended at the hip joint and the vessels are pulled downward. The junction of the saphenous and femoral veins must exert traction on the margins of the opening and distort it. The different margins are not subjected to the same conditions.

(*a*) The medial margin is the fascia pectinea or, at most, a few millimetres of fascia lata intervening between the aperture and the fascia of the pectineus. The pectineus muscle, being a flexor of the hip joint, is elongated on extension and moves downward, carrying its fascia with it. This decreases the tension on the medial margin but does not entirely eliminate it. This area is close to the origin of the muscle and the range of muscle movement must be small, so that there is a distinct pull on the fascia, which can usually be seen to be pulled into a fold that forms the immediate medial margin of the opening.

(*b*) The lateral border is attached to the inguinal ligament near its medial end, a relatively unyielding anchorage, and the fascia lata must bear almost the full strain. Hence, the firm, very distinct character of this margin.

(*c*) The inferior edge, lying in the angle between the saphenous and femoral veins, is the part actually pulled upon and is thickened and sharply defined.

(2) A new series of adult measurements appears in Table I. These were made at the time of the first exposure and are free from any error due to shrinkage. The figures recorded represent the relation, in millimetres, to the line joining the pubic tubercle and the anterior superior iliac spine. The femur lengths are given only to the nearest half-centimetre.

Table I

Subject	Side	Origin of deep circumflex iliac artery	Origin of inferior epigastric artery	Junction of saphenous and femoral veins	Femur length
1	R	−23	−16	−66	435
	L	0	− 3	−55	
2	R	−26	−15	−53	420
	L	−18	−14	−56	
3	R	−16	− 1	−42	390
	L	−16	1	−38	
4	R	− 9	− 7	−50	395
	L	− 9	− 2	−60	
5	R	− 3	− 4	−52	410
	L	*	1	−57	
6	R	−11	− 3	−67	425
	L	−16	− 3	−61	
7	R	24	19	−37	425
	L	20	17	−41	
8	R	− 5	0	−58	425
	L	3	6	−51	
9	R	− 5	− 6	−51	395
	L	− 7	− 5	−45	
10	R	− 6	− 4	−63	465
	L	− 2	− 4	−56	
11	R	− 9	− 1	−57	450
	L	− 8	− 8	−60	
12	R	6	7	−58	380
	L	0	2	−55	

* There were two deep circumflex iliac arteries and this side is omitted from all calculations.

Table II. *Foetal measurements*

Subject	Side	Origin of deep circumflex iliac artery	Origin of inferior epigastric artery	Junction of saphenous and femoral veins	Femur length
1	R	2	3	−6·5	78
	L	2	4	−6	
2	R	2	1	−6	53
	L	2·5	3	−5	
3	R	0	2	−9	70
	L	1	2	−9	
4	R	1	2·5	−4·5	52
	L	1·5	3·5	−6·5	

From Table I:

Average level of deep circumflex iliac = − 5·62
Average level of inferior epigastric = − 1·91
Average level of junction of veins = − 53·7
Average femur length = 418

From Table II:

Average level of deep circumflex iliac = 1·5
Average level of inferior epigastric = 2·5
Average level of junction of veins = − 6·56
Average femur length = 63·25

$$\text{Growth ratio} = \frac{\text{Adult femur}}{\text{Foetal femur}} = \frac{418}{63{\cdot}25} = 6{\cdot}6.$$

Expected average level of venous junction of adult = $-6{\cdot}56 \times 6{\cdot}6 = -43{\cdot}3$.
Actual average level of adult = − 53·7.
Downward movement of venous junction 53·7 − 43·3 = 10·4 mm.

Now let us consider the arteries:

Expected deep circumflex iliac of adult = $1{\cdot}5 \times 6{\cdot}6 = 9{\cdot}9$.
Actual average level = − 5·62.
Downward movement = $9{\cdot}9 + 5{\cdot}62 =$ **15·52** mm.
Expected inferior epigastric of adult = $2{\cdot}5 \times 6{\cdot}6 = 16{\cdot}5$.
Actual average level = − 1·91.
Downward movement = $16{\cdot}5 + 1{\cdot}91 =$ **18·41** mm.

$$\text{Average downward movement} = \frac{15{\cdot}52 + 18{\cdot}41}{2} = \mathbf{16{\cdot}96} \text{ mm.}$$

But downward movement of venous junction is only 10·4 mm.
The vein has been retarded 16·96 − 10·4 = **6.56** mm.

It is suggested that this failure of the venous junction to descend to the same extent as the arteries is due to the resistance of the fascia lata and that the pull of the descending veins is the actual mechanism responsible for the formation of the adult saphenous opening.

During the descent, the saphenous opening is elongated and the fatty superficial fascia is pressed into the aperture, where it becomes condensed to form the cribriform fascia, a structure with no just claim to the term "fascia".

(3) One may well ask, "Why does the lateral margin have the typical curve, if formed by a straight downward pull?" For one thing, it must accommodate itself to the cylindrical vein. But a more potent factor is the lateral pull of structures piercing the fascia lata at the lateral side of the opening. This influence can be shown to be an important one. One may frequently see a very distinct curved line, continuous with the inferior margin of the saphenous opening at its pectineal attachment, but curving upward toward the inguinal ligament to enclose a much larger area. The superior end, also, is usually closely related to the inguinal end of the lateral margin of the saphenous opening, but that margin is not particularly distinct. Some sign of this larger curve may be observed in at least 50 % of specimens and is very well developed in 15–20 %. The greatest development is reached in those subjects where the superficial circumflex iliac and the superficial epigastric arteries pierce the fascia lata by a common trunk, lateral to the saphenous opening. The vessels may be seen hooking sharply about the curved ridge and there is convincing evidence that the tension of the common trunk is responsible for the fascial thickening. The fascia lata within this wider arc is thin and poorly developed.

(4) The deep external pudendal artery usually passes through the angle between the saphenous and femoral veins and must be pulled downward. This artery often pierces the fascia of the pectineus muscle, immediately medial to the vein, and the aperture is commonly in the form of a vertical slit, up to 15 mm. in length, with the artery lying in its inferior end. This certainly suggests traction.

(5) There is a definite relation between the levels of origin of the two arteries and the level of the venous junction. The average level of the union of the two veins is −55·87 mm. in those specimens where the origin of the inferior epigastric artery is below the base line, but only −46·66 mm. in those above that line. For the deep circumflex artery, the figures are −55 and −48·8 mm.

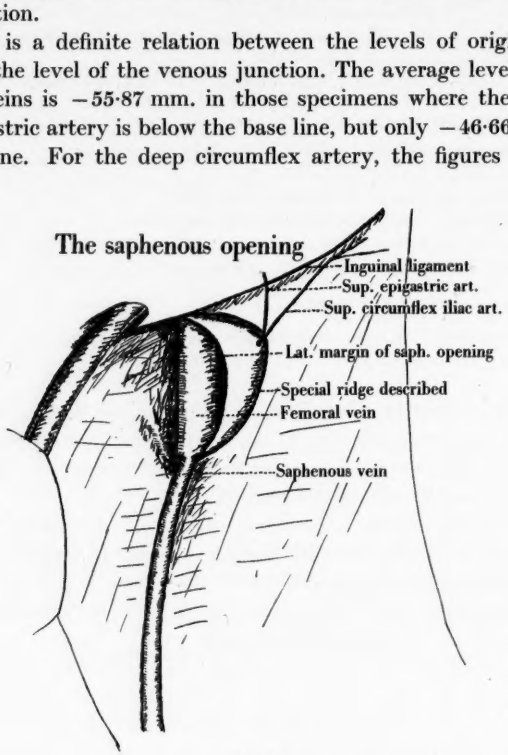

Fig. 1.

(6) Further experience confirms the impression that, as a general principle, the lower the junction of the saphenous and femoral veins, the more sharply defined and highly developed is the inferior margin of the opening. Exception must be made of those that show the secondary ring described in (3).

It is freely admitted that there are possibilities of error in the statistical arguments presented. We do not know the level of the maximum downward movement, nor how far it extends, either superiorly or inferiorly. Nor need the growth ratio be the same at all points, although we have calculated the vein according to the range of movement established for the artery. There is evidence to suggest that the actual growth ratio of the arteries is considerably less than that used in the computations. The deep circumflex iliac artery arises

at a lower level than the inferior epigastric, the difference averaging 1 mm. in the foetus and 3·71 mm. in the adult. This suggests a growth ratio of only 3·71, even ignoring the possibility of any stretching. With a lower ratio, the downward movement must be greater and the retardation of the vein more than shown. These factors can be appreciated but I know of no means by which they may be either overcome or measured. It is interesting to note that, in the series in which some shrinkage had occurred, the average distance between the two arterial origins was 3·25 mm., a figure so close to our second average of 3·71 that it suggests a fair degree of reliability for the calculations.

Although one very suggestive case was seen, our material has not permitted a study of any possible relation between the inferior margin of the saphenous opening and the clinical condition of varicose veins. It does not appear too presumptuous to suggest that such a fascial band might cause venous obstruction, especially on extension of the thigh. One would hope that such might be true as a very easy surgical procedure would then be available.

SUMMARY

It is suggested that the peculiar and characteristic structure of the saphenous opening in the fascia lata is due to the downward movement of the venous junction, itself the result of adjustments made necessary by the extension of the hip joint after intra-uterine development in the position of acute flexion. Only the dissection of infants of various ages will tell us how long a time is required for the vessels to become stabilized in their final positions. The adjustment almost certainly is not an immediate one.

The statistical evidence is not necessarily infallible, but it is comforting to note that it points in the same direction as the several other observations advanced to support the hypothesis.

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ANATOMICAL NOTES

PERSISTENT OPENING OF ANTERIOR CLOACAL DEPRESSION

By M. AMIN

Assistant Professor of Anatomy, Cairo

The condition of parts described in this paper seems worthy of record. The subject was a 12-year-old girl, examined by me in March 1937. Physical examination revealed complete absence of a perineal anus (Fig. 1, *10*), well-formed labia majora et minora (Fig. 1, *5* and *3*), an intact but abnormally situated hymen (Fig. 1, *9*), and an anomalous anal orifice (Fig. 1, *8*) opening backwards from the vulval cleft and partially overlapped by the hymen. Through this anomalous anal opening the bowel discharged involuntarily. The urinary meatus (Fig. 1, *4*) and clitoris (Fig. 1, *2*) are normal, likewise the vaginal orifice (Fig. 1, *6*); the posterior vaginal wall is interrupted by the unusual termination of the gut, the thick margin of tissue between this anomalous aperture and the vaginal opening is somewhat suggestive of the embryonic septum which divides the cloaca into rectum and urogenital sinus.

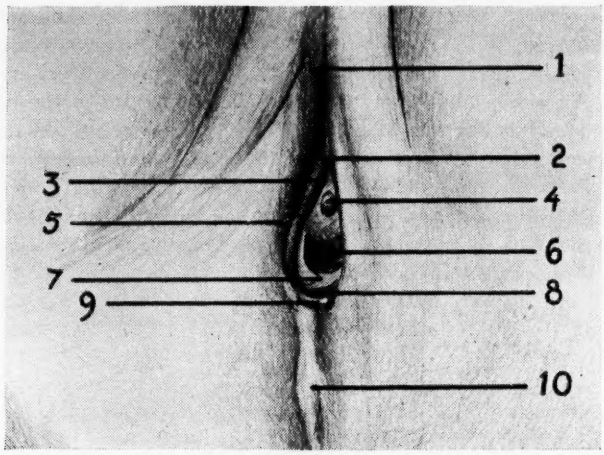

Fig. 1. Anomalous anal termination, showing (*1*) mons veneris, (*2*) clitoris, (*3*) labia minora, (*4*) urinary meatus, (*5*) labia majora, (*6*) vaginal orifice, (*7*) septum between vaginal and abnormal anal orifices, (*8*) abnormal anal opening, (*9*) fold regarded as hymen, (*10*) usual site of anus, imperforate and prominent.

The habitual involuntary discharge of bowel contents through this vaginal anus supports the views enunciated by Popowsky (1899) concerning the development of the perineal sphincteric musculature. Popowsky held that the m. sphincter ani externus and the m. sphincter sinus urogenitalis both derive from a common subcutaneous primordium, and that their differentiation is entirely dependent upon complete division of the cloacal aperture into anal and urogenital orifices. The absence, in the present case, of a perineal anus presumably prevented such differentiation,

recalling the condition of affairs typical of certain marsupials, where anal and urogenital apertures, opening into an ectodermal cloaca, are guarded by a common sphincter. Defective growth of the cloacal septum is held responsible for the present malformation: the septum has failed to reach the cloacal membrane, for had it done so either a normal or an imperforate anus would have resulted. The septum, and with it the anal canal, has grown forwards instead of backwards and so the latter has opened directly through the posterior wall of the urogenital sinus.

A similar case recorded by MacKenzie (1906) in a much younger child was interpreted by Robinson as an example of "anal canal opening into a cloaca": Keith (1933) regards such cases as examples of the "ancient cloacal orifice of the rectum". Cases described by Reichel (1888) and others, however, differ somewhat from the present one: in them the rectum terminates normally, but a tendency towards an anterior cloacal termination is evidenced by a small canal connecting the rectum with the vestibule immediately behind the hymen. Somewhat similar are those further cases possessing a small but normally situated anal orifice, wherein the rectum communicates with the vestibule or the male urethra. In all such, a defective cloacal septum plays a causative part.

The curious position, in the present instance, of the fold regarded as hymen (Fig. 1, *9*) is noteworthy, for its disposition might be held to suggest its derivation from the urogenital sinus only, or from surface mesoderm in good part. This view is supported by cases recorded by Pozzi (quoted by Wood Jones) which boasted a hymen but no vagina (the latter being represented by a small blind pocket derived from the urogenital sinus), and by cases described by Wood Jones (1927) of (*a*) single hymen with double vagina, (*b*) single hymen common to vaginal and urethal orifices. According to Wood Jones (1927) the hymen is formed from the mesoderm interposed between the surface and the bulbar apex of the downgrowing vaginal cords. Bloomfield and Frazer conclude, however, that the hymen is produced by the contact of vaginal and urogenital sinus structures. The presumptive hymen in the case herein detailed lies above the anomalous anal orifice, and thus separates it from the vaginal aperture: it is therefore unrelated to the contact site of Mullerian tubercle and urogenital sinus. Its attempted interpretation reveals a divergence of views concerning hymeneal development and emphasizes the need for further research in this matter.

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THE SAPHENOUS ARTERY

By Y. H. AASAR
Faculty of Medicine, Cairo

This case has been found in the right lower limb of an adult dissecting room subject. Fig. 1 shows that the arteria genu suprema (*A.G.S.*), arising as usual from the distal end of the second part of the femoral artery, giving off almost immediately its musculo-articular branch and continuing as a well-marked saphenous artery. This descends in the specimen with the saphenous nerve on the medial side of the lower part of the thigh and the upper half of the leg. It is tortuous in its lower half at the end of which it pierces the fascia attached to the medial border of the tibia. Gaining the posterior compartment of the leg, it ascends for a little distance and joins the

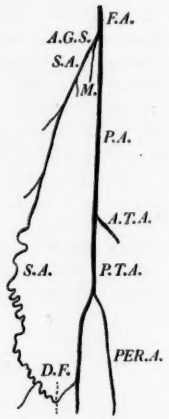

Fig. 1. Drawing to show the saphenous artery seen in the case here recorded. *A.G.S.* arteria genu suprema; *M.* musculo-articular branch; *F.A.* femoral artery; *P.A.* popliteal artery; *A.T.A.* anterior tibial artery; *P.T.A.* posterior tibial artery; *D.F.* deep fascia; *S.A.* saphenous artery.

posterior tibial artery. Apart from its tortuosity, the vertical distance between its beginning and its termination is 30 cm. Numerous branches are given from it on the front of the leg but none of them could be followed to the dorsum of the foot.

In all primates except man a saphenous artery accompanies the saphenous nerve and ultimately forms the dorsalis pedis artery. Keith states that at no stage of human development does the saphenous artery serve as a main channel although the superficial branch of the anastomotica magna (which represents this vessel in man) is more highly developed in the eighth week than at any subsequent period.

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A LEFT SUPERIOR VENA CAVA DRAINING THE BLOOD FROM A CLOSED CORONARY SINUS

By ADRIAN F. REED

Department of Anatomy, Tulane University of Louisiana

THESE anomalies occurred in the cadaver of a white male drug addict, aged 77. The cause of death was given as pulmonary oedema.

The left superior vena cava (Fig. 1) extended from the left end of the coronary sinus to the left innominate vein at a point 1·5 cm. from the origin of the latter vessel. Its length was 11·5 cm. The diameter at the cephalic end was 3 mm., at the middle 4 mm., and the caudal end enlarged to a diameter of 7 mm.

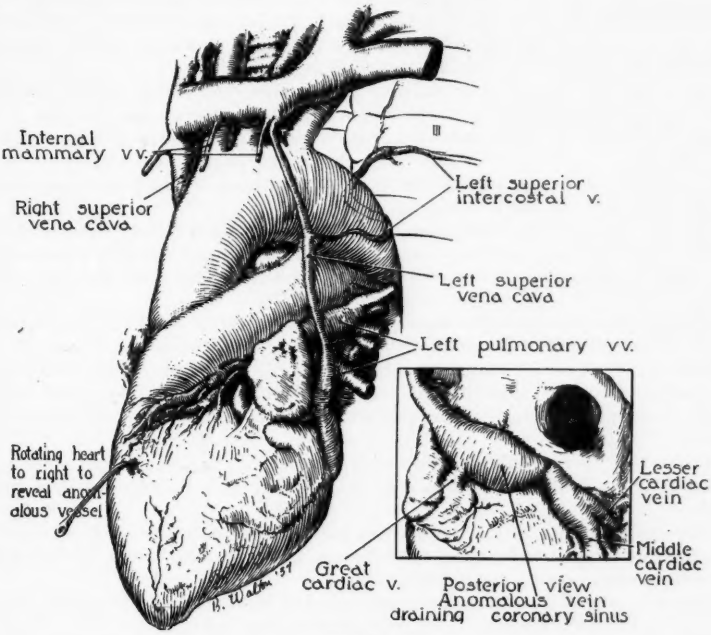

Fig. 1.

Two veins emptied into this vena cava in the upper part of its course. The higher vessel was small and terminated just below the junction of the left superior vena cava and the left innominate vein. Two small tributaries joined this vein. One of them drained the area immediately above the aortic arch between the left common carotid artery and the left subclavian vein. The other one was closely applied to the phrenic nerve. The lower of the two veins flowing into the anomalous vena cava drained the

second, third, and part of the fourth intercostal spaces. It was evidently the left superior intercostal vein. Anastomoses with the vena suprema were found and caudally the vein was continuous with the accessory hemiazygos vein. It terminated at the level of the upper part of the fourth thoracic vertebra.

Sections were made of the lower end of the left superior vena cava. The walls of this vessel resembled histologically that of any other vein of comparable size except for a layer of cardiac muscle which clothed it for about 1·5 cm. beyond the normal extent of the coronary sinus.

The coronary sinus, from its external appearance, seemed to be normal except for an increase in size and its connexion with the left superior vena cava. Upon examination of the inner surface of the right atrium no opening from the coronary sinus could be found. The sinus was dissected entirely free from the atrial wall. Histological preparations from sections of the atrial and sinus walls in the region usually occupied by the ostium of the sinus were made. These showed no scar tissue or other evidence of pathological conditions. Both areas had the normal characteristics of their respective tissues.

The presence of a left superior vena cava is not a rare anomaly. Beattie (1931) in presenting three cases of his own stated that the total number recorded was 175. Since that time Chlyvitch (1932), Kodama (1931), Seshachalam & Nagendran (1933), Papez (1938) and others have made additions which increase the total to 188 cases in man. Grant (1917) described a left superior vena cava in a cat. Chase & De Garis (1938) found a similar vessel in an orang-outang.

However, the absence of the opening between the right atrium and the coronary sinus is very infrequent, therefore the reporting of this case seemed justified. Le Cat (1738) was the first to record this type of variation in the vascular system. According to a quotation made by Marshall (1850) he found, in a child eight days old, the coronary veins united in a single trunk which, without entering the right auricle, emptied into the left subclavian vein.

Gruber (1885), Siding (1896), Nabarro (1902), Beyerlein (1914), and Harris *et al.* (1927) have described cases in which the blood flow in the coronary sinus and the left superior vena cava was reversed, due to the absence of an atrial opening from the sinus.

Hutton (1915) found a heart having a closed coronary sinus with an oval depressed area in the atrial wall of the sinus at the normal site of the opening between the sinus and atrium. The floor of this area had several pit-like markings. Opposite this region the inner surface of the atrium displayed a circular shallow depression which was also grooved and pitted. None of the pits formed a connexion between the sinus and atrium.

Peele (1932) presented a case in which the coronary sinus and right atrium communicated by a channel so small as to be judged inadequate to carry any large amount of blood.

One case, in a cat, of a superior vena cava sinistra carrying coronary blood was described by Grant (1917). Here the coronary sinus was found to be short and failed entirely to connect with the right atrium.

The presence of a left superior vena cava has been generally described as a persistence of the early embryonic left common cardinal vein.

Various causes have been ascribed to the formation of a closed coronary sinus. Beyerlein, according to Grant (1917), suggested two possibilities: closure as a result of endocardial inflammation, or, by mechanical means resulting from decreased pressure in the left superior vena cava after the formation of the left innominate vein and increased pressure in the right atrium from the inferior vena cava and right superior vena cava.

Hutton (1915) likewise makes two suggestions: that the septum between the sinus and atrium resulted from the fusion of the coronary parts of the right and left venous

valves, or fusion of a very large Thebesian valve with the edges of the opening from the sinus.

Hutton's latter suggestion may be the solution as to the way in which the sinus opening was closed in the case described in this paper. If so, the fusion must have taken place in a very early stage as no evidence of the union can be demonstrated histologically. The section of tissue removed from the site of the normal opening was carefully examined and was found to be structurally identical with other samples from the wall of the sinus.

SUMMARY

A case of a left superior vena cava and a closed coronary sinus with reversal of cardiac venous blood flow is described. A suggestion is made concerning the mechanism resulting in the closure of the opening between the right atrium and the coronary sinus.

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REVIEWS

A Monograph on Veins. By KENNETH J. FRANKLIN, D.M., M.R.C.P. (London: Baillière, Tindall and Cox.) 1938. Pp. xxii + 410, 45 figs. Price 27*s.*

In this monograph the survey of literature and the manifold aspects of the subject are given extensive treatment, and enlivened by a historical outlook which comes from a real enthusiasm for the history of science. It is probably true that never before have so many scattered but important scientific publications on this subject been brought together in the 350 pages of this monograph.

After the historical introduction there follows an account of the embryology of veins written by Mr K. Richardson. This chapter manages to retain principle and simplicity amid the complexity of embryonic venous trunks which embryologists have thought fit to recognize. Then follow some very stimulating chapters dealing with the microscopic structure of veins. These show the fruitful result of correlating structure with function. But there are statements here which must be received with caution since the author seems to be favourably inclined towards the theories of syncytial innervation of veins.

The author is to be congratulated on his treatment of the recent work on arterio-venous anastomoses. Paragraphs on venous valves are well done, not only because the full evidence modifying v. Bardeleben's laws is indicated, but also for the selection from modern work on direct observation of the living vessels. The chapter on blood depots is a useful summary of a large field of research. The accounts of the mechanical conditions of flow, pressure, etc., do make clear what is and what is not established. The chapter on fluid interchanges and nervous chemical control are full expositions. The application of these basic scientific facts to the clinical problems concludes the book, and in this the advances in technique suitable for clinical work have been included.

Altogether it is a standard monograph which for some time will serve as the work of reference on this subject, and which in its co-ordinating principle is in harmony with the general trend of modern biological science.

Surgical Anatomy of the Head and Neck. By J. F. BARNHILL, M.D., F.A.C.S., LL.D. $7\frac{1}{2} \times 10\frac{1}{4}$. (Baillière, Tindall and Cox.) Pp. xiv. + 922, 431 figs. (many in colour). Price 90*s.*

This volume, which is published by subscriptions, will be of great interest to the author's former pupils. It consists of a series of freely transcribed lecture notes on the Surgical Anatomy of the Head and Neck, which the author presumably compiled during his years of teaching. There are some 400 illustrations at the end of the book the majority of which are crudely diagrammatic.

A Method of Anatomy. By J. C. B. GRANT, M.C., M.B., Ch.B., F.R.C.S. 10×7. (Baillière, Tindall and Cox.) Pp. xx + 650, 564 figs. Price 27*s.*

This book is readable and brief, the illustrations are diagrammatic and uncomplicated. Beginners in Anatomy who find topographical anatomy difficult to grasp will welcome this book.

Histological Technique, for normal tissues, morbid changes and the identification of parasites. By H. M. CARLETON. (Oxford University Press.) 2nd ed. 1938.

An acquaintance with histological methods is a necessity for most biological research workers whether they are required to carry out their own technical work or to instruct others to do it for them. We consider this book to be a valuable guide for those commencing to gain experience in a subject which is full of contradictions, arbitrary statements and empirical procedures. It summarizes the more important information which is scattered in the literature and in several larger works on histological technique.

The 2nd edition has been improved by the addition of some recent imbedding and staining methods, but we should like to point out that, in the chapter dealing with methods for special organs, tissues and cell components, the methods advised are sometimes not typical of those used by the experts in any particular field. Methods for the investigation of the anterior lobe of the hypophysis, of the islets of Langerhans and the organ of Corti, for instance, all of which have been improved very considerably in recent years in this country or elsewhere, are omitted or replaced by techniques of doubtful value.

Physikochemische Grundlagen der Histologischen Methodik. By K. ZEIGER. (Theodor Steinkopf, Dresden and Leipzig.) 1938. Pp. xi+202. Price R.M. 14.

This book consists of a review of the literature—mostly German—on the physics and chemistry of histological processes. The subjects discussed include fixation, staining, metallic impregnation, vital staining, and special procedures such as incineration.

Both the theory and practice of these methods are considered, special attention being paid to the interpretation of fixing and staining phenomena in the light of colloid chemistry.

Meetbare Variabelen van den menschelijken Schedel en hun onderlinge Correlaties in Verband met Leeftijd en Geslacht. (*Measurable Variables of the Human Skull and their intercorrelations in Connexion with Age and Sex.*) By A. DE FROE. 1938. Amsterdam. Pp. 79+18 tables.

This thesis is concerned with the measurements of a series of 205 male and 90 female skulls belonging to subjects of known age and stature. The skull capacity and 31 rectilinear measurements and arcs were ascertained in each subject. The usual indices, modes, averages, standard deviations, coefficients of variability are given. The correlations with age, stature and the sexual differences were determined. The thirty-nine conclusions are expressed in Dutch, German and English.

OBSERVATIONS ON THE MORPHOLOGY OF THE HUMAN TONGUE

By SHAFIK ABD-EL-MALEK, M.B., B.Ch.

Anatomy Department Medical Faculty, Cairo

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INTRODUCTION

In another paper (1937) the musculature of the tongue in a series of animals (cat, rat, and dog) has been studied both experimentally and histologically with regard to the action as well as the attachment of the individual muscles.

In this communication it is intended to correlate our findings on animals with those present in the human tongue; for a detailed description of the architecture of the tongue is not available in the literature.

Though it is an important organ, yet it has not, so far, been given an equal share in the detailed anatomical studies allotted to other organs in the body.

The intricate interlacement of the fibres of its muscles, and the difficulty or, rather, the impossibility of complete separation of individual muscles may be admitted as a possible clue to such lack of study.

MATERIAL AND METHODS

The observations here recorded comprise the description of both the membranous and muscular structures as well as the nervous and vascular supply of the human tongue. They were made by the help of the dissecting microscope, celloidin transverse serial sections of human tongue, and coloured celloidin injection into the vessels.

It is more helpful to deal first with the description of the stroma or connective tissue framework of the tongue as this not only separates the different muscles and includes most of the nerves and vessels of the organ, but also serves as a site of attachment for these muscles.

The connective tissue framework of the tongue

This includes the tunica propria of the mucous membrane, the submucous tissue, and the fibrous septa which exist in its substance.

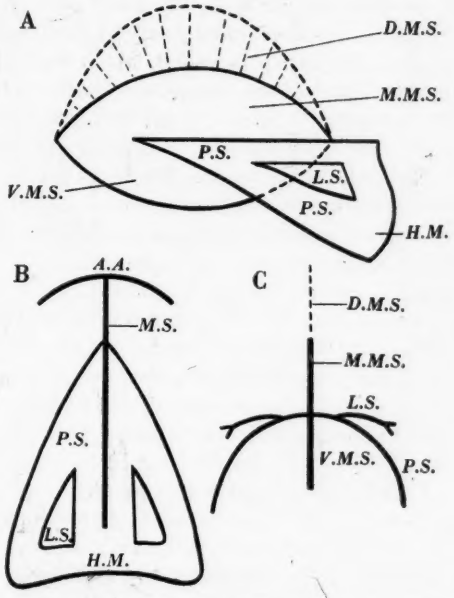
The lamina propria in the human tongue becomes gradually thicker and stronger on the dorsum of the tongue as it extends from its root to the tip, where it forms a cap-like thickening which is more extensive ventrally than dorsally. This condensed part of the lamina propria at the tip may be named the *anterior arch* (see Text-fig. 1 and Pl. I, figs. 1, 2). It gives attachment to the superior longitudinal muscle dorsally, and inferior longitudinal, styloglossus, some of the most anterior fibres of the hyoglossus and genioglossus muscles ventrally. The lamina propria can be seen in the ventral part of the tongue from the tip to about the attachment of the frenulum linguae.

Septa of the tongue. In addition to the median septum, there are two other septa in the substance of each half of the tongue, which we suggest, may be called the paramedian and lateral septa.

The median septum is well known both morphologically and anatomically. Its middle portion is the thickest and strongest part of the septum. It is clearly seen in the anterior two-thirds of the tongue. Its widest part is about its middle, and it decreases gradually both anteriorly and posteriorly. It gives attachment to the medial part of the transverse muscle, one on either side. The inferior or ventral part of the septum intervenes between the medial surfaces of the two genioglossi muscles, and is coterminous with them. It consists of a very loose areolar tissue with a fatty pad at its posterior part. Dorsally it is practically absent but can be detected between the two superior longitudinal muscles as a few strands in some sections (see Text-fig. 1 A, B and C, Pl. I, fig. 4 and Pl. II, figs. 5, 6).

The paramedian septum is the strongest septum in the substance of the tongue especially at its posterior part. It lies between the genioglossus muscle medially and the surrounding muscles both dorsally and laterally—i.e. transverse and vertical muscles dorsally, and inferior longitudinal, hyoglossus and styloglossus muscles laterally. It moulds itself on the lateral surface of the genioglossus, and consequently it is triangular in outline, with its narrow apex anteriorly reaching as far forward as and corresponding in extent with the genioglossus. Posteriorly it broadens out, assuming a more or less horizontal direction, to be attached to the hyoid bone. This part is known as the *hyoglossal membrane* (Gray's and Cunningham's *Anatomy*). Medially it is attached to the median septum at the junction of its middle and ventral parts, while its lateral surface in its posterior part gives rise to the lateral septum which will be described later. It then reaches the submucous layer and intermuscular septa of the muscles of the floor of the mouth. The lingual artery after reaching the septum is contained in its substance accompanied by veins and a very short part of the hypoglossal nerve (see Text-figs. 1 A, B and C, 2, 3 and 4, and Pl. I, figs. 3, 4 and Pl. II, figs. 5, 6).

Lateral septum. This is placed between the inferior longitudinal muscle medially, and hyoglossus and styloglossus muscles laterally. It is somewhat triangular in shape, and its apex is directed anteriorly, ending where the three muscles unite, while its base, which is directed posteriorly, is continuous with the posterior part of the paramedian septum of the hyoglossal membrane. Its medial border is attached to the paramedian septum whereas laterally it divides in its ventral part into two lamellae, lateral and medial, which intervene between the styloglossus, hyoglossus, and inferior longitudinal muscles respectively. In its substance lies the trunk of the lingual



Text-fig. 1. Diagram to show roughly the outline of the connective tissue framework of the tongue A. The three septa and their relation to one another as seen from the left side of the tongue. The dorsal part of the median septum which is occasionally seen between the two superior longitudinal muscles is indicated by short lines. B. The three septa, the hyoglossal membrane, and the anterior arch as seen in a horizontal plane are illustrated roughly and diagrammatically. C. The septa as seen in a transverse section at the posterior third of the tongue.

artery with its dorsalis linguae branches, as well as the glossopharyngeal nerve in its medial lamella. Its lateral lamella contain in its substance the hypoglossal nerve—with its branches to styloglossus, hyoglossus and genioglossus muscles. Its venae comites and the lingual nerve are also contained in its substance (see Text-figs. 1 A, B and C, 2, 3, 4 and Pl. II, fig. 6.)

Musculature of the tongue

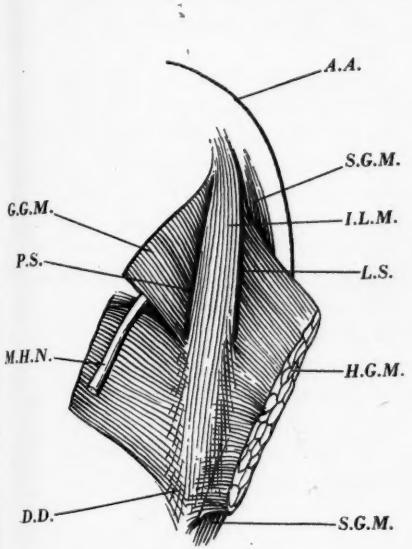
The superior longitudinal muscle, which is usually described as a thin stratum, is in reality only so in its peripheral parts, i.e. in its anterior, posterior and lateral parts. In the middle two-fourths of the tongue it is a bulky mass triangular in cross section, lying dorsal to the transversus and verticalis muscles (see Pl. I, figs. 3, 4). Its posterior fibres are reduced to a thin sheet which is attached to the lamina propria of the mucous membrane of the posterior third of the tongue. Some of these fibres can be traced backwards with difficulty to be attached partly to the lower and ventral part of the epiglottis and hyo-epiglottic ligament and partly to the hyoglossal membrane. The anterior fibres gradually flatten to a sheet, which is attached partly to the lamina propria on the dorsum of the tongue, and partly to the dorsal part of the anterior arch. Laterally the muscle is reduced gradually in thickness to join the longitudinal fibres of styloglossus, hyoglossus and inferior longitudinal muscles at the lateral border of the tongue. The fibres of the two muscles—one on each side—are in some localities separate, due to the interruption of some fibres of the two genioglossi muscles which may reach the lamina propria on the dorsum of the tongue either directly, or after decussation those of the opposite side (see Pl. I, figs. 3, 4, and Pl. II, figs. 5, 6, 7).

Inferior longitudinal muscle. This is a narrow muscle, oval in cross section, which extends between the paramedian septum and the medial lamella of the lateral septum. Posteriorly it generally possesses two attachments, i.e. medial and lateral. The medial part arises in conjunction with the most lateral and ventral fibres of genioglossus muscle—with whose fibres it decussates—from the anterior surface of the hyoid bone. Its lateral attachment is from the lateral part of the body of the hyoid bone, as well as from the root of its great cornu, together with the most medial of the decussating fibres of hyoglossus muscle (see Text-fig. 2). A few fibres in between these two attachments are occasionally seen to extend posteriorly to be attached to the stylohyoid ligament. As the muscle proceeds towards the tip of the tongue it partially rotates inferomedially. In about its middle it blends with the anterior fibres of genioglossus hyoglossus, and styloglossus muscles, with which it forms the ventral part of the tip. All these muscles are attached to the anterior arch (see Text-figs. 2, 3).

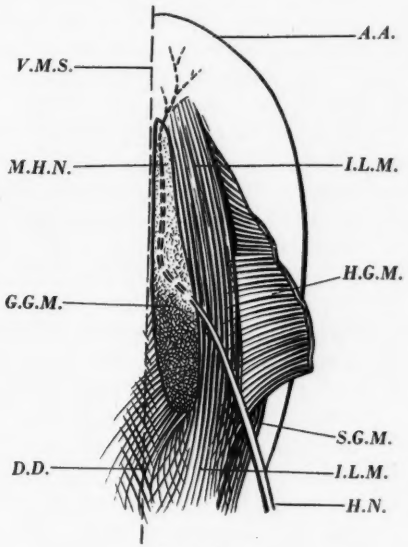
The transverse muscle forms a good part of the thickness of the tongue, especially in its middle. It is situated between the superior longitudinal muscle dorsally, and paramedian septum, genioglossus and inferior longitudinal muscles ventrally. It can be easily described as a series of lamellae which extend from the median septum laterally. The more superficial fibres take a dorsal direction, the deepest ones are directed ventrally. The muscle consists of two types of fibres, long and short. The former are long enough to reach the lamina propria of the mucous membrane of the side of the tongue, to which they also have their lateral attachment. The latter are the

majority of the fibres which are interrupted by the neighbouring muscles with their septa, to which they are naturally attached. All the muscular fibres decussate with those of the vertical muscle and some decussate with fibres of genioglossus muscle. The transverse muscle extends more anteriorly and posteriorly than does the vertical one (see Pl. I, figs. 3, 4 and Pl. II, fig. 5).

The vertical muscle consists of several bundles of fibres placed more or less in a vertical direction. They decussate intimately with the strata of the transverse muscle, thus forming together a considerable part of the central mass of



Text-fig 2.



Text-fig. 3.

Text-fig. 2. Diagram to show the intimate relation between the posterior part of the inferior longitudinal muscle with genioglossus medially and hyoglossus muscle laterally. Anteriorly the same muscle (i.e. the inferior longitudinal muscle) interdigitates with the most anterior fibres of the genioglossus medially, and hyoglossus and styloglossus muscle laterally in forming together the anterior part of the tongue.

Text-fig. 3. Diagram illustrating the course of the hypoglossal nerve throughout its whole course in the substance of the tongue. It is seen in a dissection from its ventral aspect.

the tongue. They consist of two sets of fibres, a few long fibres which reach the lamina propria dorsally, or the submucous layer ventrally. The rest of the fibres fall short between the paramedian septum and adjacent muscular septa (see Pl. I, figs, 3, 4 and Pl. II, fig. 5).

Genioglossus muscle. This is an extensive muscle which constitutes the main bulk of the tongue posteriorly. It is present in all transverse and horizontal sections, and can be seen in all parasagittal sections except those situated most laterally. It arises by a short musculo-tendinous origin from the superior

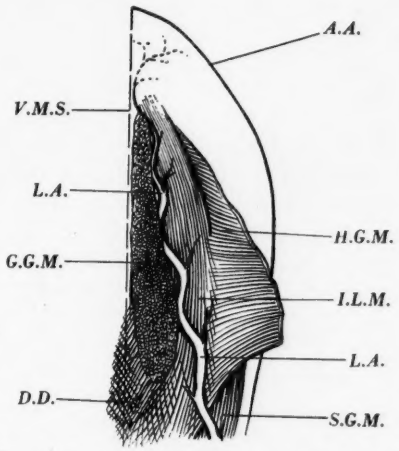
mental spine on the inner surface of the symphysis menti, and spreads dorsally in the substance of the tongue from root to tip, in a fan-shaped radiation both antero-posteriorly and medio-laterally. Its anterior fibres run in a curved antero-dorsal direction to join the anterior fibres of inferior longitudinal, hyoglossus, and styloglossus muscles, with which it is attached to the anterior arch (see Text-fig. 2). Its posterior fibres run horizontally and backwards to the root of the tongue to the anterior surface of the hyoid bone and anterior surface of the base of the epiglottis. The intermediate fibres diverge with different degrees of obliquity between the two above-mentioned parts to be attached to the ventral surface of the paramedian septum, and the lamina propria of the mucous membrane on the dorsum of the tongue.

Most of its fibres, especially those of the intermediate and posterior parts of the muscle, radiate in a medio-lateral direction in addition. The lateral fibres diverge in an outward direction. The medial fibres decussate with those of the opposite side posteriorly, whereas the anterior fibres pass nearly vertically and dorsally. The middle fibres run dorsally with different degrees of obliquity in between the above mentioned two parts. Some of the medial dorsal fibres of the same muscle interdigitate with the transverse muscle before they can escape dorsally to the mucous membrane of the dorsum of the tongue. A good number of these escaping fibres, before they reach their termination will either interdigitate with the fibres of the superior longitudinal muscle of its corresponding side, or pass in between these two muscles. Some of these latter fibres decussate with the corresponding fibres of the opposite genioglossus muscle before being attached to the lamina propria (see Pl. II, figs. 5, 6, 7). At the posterior part of the posterior third of the tongue, where the superior longitudinal muscle is reduced to a thin sheet of muscular fibres, the genioglossus interdigitates with it directly. At the same time it lies in close approximation to the dorsum of the tongue to which they both are attached (see Text-figs. 2, 3, 4, Pl. I, figs. 3, 4 and Pl. II, figs. 5, 6, 7).

Hyoglossus muscle. This is a quadrangula rmuscle in its lower two-thirds but radiates in a fan-shaped manner in its upper third. It lies between inferior longitudinal and genioglossus muscles medially, and styloglossus, mylohoid muscles, and submaxillary gland laterally. It arises from the lateral part of the anterior surface of the body of the hyoid bone—in conjunction with the lateral fibres of the inferior longitudinal muscle as well as from the whole extent of the greater cornu. The anterior part of the muscle, i.e. that portion which arises from the body of the hyoid bone, lies superficial to the part arising from the greater cornu, and interdigitates at its origin with superficial and deep fibres of the geniohyoid.

The proximal third of the muscle consists of fibres, which, in addition to their radiation antero-posteriorly, run medially and dorsally. The anterior fibres run nearly longitudinally towards the tip of the tongue to which they are attached. Its posterior fibres, which lie under cover of styloglossus with which they decussate, radiate nearly transversely and posteriorly towards the

root of the tongue, decussating again with the lateral part of the inferior longitudinal muscle medially (see Text-figs. 2, 3, 4). The middle fibres, which run more medially and dorsally, differ in their obliquity between the anterior and posterior fibres. Both the middle and posterior fibres are partly attached to the paramedian septum and partly after decussation join the fibres of superior longitudinal muscle dorsally and transverse and genioglossus muscle ventrally (see Pl. II, figs. 5, 6, 7).



Text-fig. 4. Diagram to show the relation of the lingual artery to the lingual muscles as they are dissected from the ventral surface of the tongue. The decussation between the posterior parts of the two genioglossi is shown in the median plane.

The styloglossus muscle arises from the anterior and lateral surface of the styloid process near its apex, and from the proximal part of the stylomandibular ligament. It is flattened medio-laterally to begin with. As it runs downwards and forwards towards the tip of the tongue, it divides into two parts, lower and upper. The former is the shorter and smaller of the two and ends by decussating superficially with the lateral surface of the hyoglossus muscle. The latter is the larger part and proceeds towards the tip of the tongue where its deep fibres interdigitate with the same muscle. At the anterior border of hyoglossus the muscle is enlarged in a ventral direction and winds round the lateral and ventral parts of the tongue until it gains its inferior surface (i.e. its inferior border becomes medial and its medial surface now looks dorsally). It then insinuates itself among the anterior fibres of genioglossus, hyoglossus and inferior longitudinal muscles, joining together to be attached to the anterior arch (see Text-fig. 2 and Pl. II, figs. 5, 6, 7).

Hypoglossal nerve

In the submaxillary region the hypoglossal nerve passes forwards, first between the submaxillary gland and hyoglossus muscle, and then between mylohyoid and hyoglossus muscles, where it gives medial branches to geniohyoid, lateral ones to the deep surface of styloglossus and superficial surface of hyoglossus, in addition to medial twigs supplied to genioglossus.

At the anterior border of hyoglossus, the nerve trunk winds upwards and medially round it, supplying several branches to its deep surface. The nerve then divides into two divisions. The smaller, which we may call the lateral division, runs in between hyoglossus, styloglossus and inferior longitudinal muscles supplying them with several fibres. The larger medial division crosses beneath the inferior longitudinal muscle, and sometimes passes through its lower fibres. It traverses the paramedian septum to gain the lateral surface of the genioglossus muscle, and follows a sinuous oblique course dorsally, medially and forwards through nearly the whole thickness of the muscle. It then reverses its course, running laterally upwards and anteriorly and distributing several twigs both medially and laterally to the genioglossus; then dorsally to the transverse, vertical and superior longitudinal muscle. Some nerve fibres were traced in company with their corresponding branches of the lingual artery along the fibres of the genioglossus to the superior longitudinal muscle. Some others were seen to supply the transverse and verticalis before reaching the superior muscle. Just before the tip the nerve terminates by giving several end branches to radiate in an arciform manner, paramedially, to the adjacent muscles (see Text-fig. 2, 3, and Pl. I, figs. 3, 4, and Pl. II, figs. 5, 6).

Lingual artery

As the lingual artery appears from the anterior margin of the hyoglossus muscle, it gives its sublingual branch. It then proceeds in a very sinuous course anteriorly, upwards, and slightly medially in the substance of the paramedian septum between the genioglossus muscle medially and inferior longitudinal muscle laterally in its course to reach the dorsum of the tongue. It forms about four to five arches dorso-ventrally, from which arteries are distributed medially, laterally and dorsally. Near the tip of the tongue it divides into its two terminal branches, medial and lateral. The former is a short and sinuous branch which runs towards the septum, anastomosing with the corresponding branch of the opposite side. The latter forms an arch to furnish its terminal twigs, some of which anastomose with its sublingual branch (see Text-fig. 4).

CONCLUSIONS

1. Besides the median septum, two other septa are demonstrated in the substance of each half of the tongue. They not only separate the different surrounding muscles, but also serve as a site of attachment for them. In

addition, they contain in their substance the trunks of the lingual vessels and nerves as well as parts of their main branches.

2. An anterior arch is described at the tip of the tongue for the attachment of the superior longitudinal muscle dorsally, and the inferior longitudinal, hyoglossus, styloglossus and genioglossus muscles ventrally.

3. The inferior longitudinal muscle has a double bony attachment to the hyoid bone in conjunction with genioglossus and hyoglossus.

4. The superior longitudinal muscle, which is demonstrated to be a bulky muscle, triangular in cross section, is indirectly attached to the hyoid bone.

5. The intralingual course of the lingual artery and the hypoglossal nerve are described as well as their main branches.

ACKNOWLEDGEMENTS

My sincere acknowledgements are due to Prof. D. E. Derry for his guidance and criticism, and to Dr Amin Bey and Dr N. Fahmy for their helpful advice. I am indebted to Mr N. Strekolovsky, the Faculty artist, for the drawings.

EXPLANATION OF PLATES I AND II

Plate I

Fig. 1. Microphotograph from a transverse section of human tongue at its tip, showing a dense mass of fibrous tissue forming a cap which covers the tip of the tongue both dorsally and ventrally and into which the longitudinal muscle fibres are attached.

Fig. 2. Microphotograph from a horizontal section at the tip of the tongue showing in a horizontal plane the thickness of the anterior arch which gives attachment to the longitudinal muscle fibres.

Fig. 3. Microphotograph from a transverse section at the anterior fourth of the human tongue. The section is mainly of longitudinal muscle fibres surrounding a narrow zone of transversely running fibres of the transverse muscle with the beginning of the middle part of the median septum.

Fig. 4. Microphotograph from a transverse section through the anterior third of the tongue. The transverse muscle, interdigitating with the vertical muscle, forms the central part of the tongue. The middle part of the median septum is seen to give attachment to the transverse muscle. No septum is seen between the two superior longitudinal muscles. The paramedian septum lies between the genioglossus muscle and inferior longitudinal muscle.

Plate II

Fig. 5. Microphotograph from a transverse section about the middle of the tongue. The transverse and vertical muscles form together about the middle third of the substance of the tongue. The median and paramedian septa are seen with the structures they contain. The styloglossus cannot be distinguished from the hyoglossus. The inferior longitudinal muscle is seen partially blending with the latter two muscles, but is separated from the genioglossus muscle by the paramedian septum.

Fig. 6. Microphotograph from a transverse section through the posterior third of the tongue. The ventral part of the median septum, containing the pad of fat, is seen at its maximum extent. The genioglossus muscle occupies more than half of the substance of the tongue. The paramedian septum separates the muscle from the inferior longitudinal muscle which can be seen only medially and ventrally. The hyoglossus muscle can be seen with the two laminae of the lateral septum both medially and laterally. The superior longitudinal muscle is reduced to a sheet which is interrupted by several bundles of the genioglossus muscle.

Fig. 7. Microphotograph from a transverse section through the tongue at the level of the hyoid bone. The medial decussating fibres of the two genioglossi muscles are clearly seen. Superior longitudinal muscle is further reduced to a few longitudinal bundles scattered among the fibres of the genioglossus muscle. The hyoglossus muscle is seen in relation to the greater cornu of the hyoid bone. The lingual artery lies deep to it, whereas the hypoglossal nerve lies laterally. The styloglossus muscle is situated in the upper lateral angle of the section, interlacing with the hyoglossus muscle. Sections of the body as well as the great cornu of the hyoid bone are seen in the ventral part of the section.

ABBREVIATIONS

H.N.	Hypoglossal nerve.
M.H.N.	Medial division of the hypoglossal nerve.
L.H.N.	Lateral division of the hypoglossal nerve.
L.A.	Lingual artery.
S.A.	Sublingual artery.
S.L.M.	Superior longitudinal muscle.
I.L.M.	Inferior longitudinal muscle.
T.M.	Transverse muscle.
V.M.	Vertical muscle.
G.G.M.	Genioglossus muscle.
H.G.M.	Hyoglossus muscle.
S.G.M.	Styloglossus muscle.
G.H.M.	Geniohyoid muscle.
A.A.	Anterior arch.
M.S.	Median septum.
V.M.S.	Ventral part of the median septum.
M.M.S.	Middle part of the median septum.
D.M.S.	Dorsal part of the median septum.
P.S.	Paramedian septum.
L.S.	Lateral septum.
H.M.	Hyoglossus membrane.
P.T.	Palatine tonsil.
P.F.	Pad of fat.
M.G.	Mucous glands.
S.G.	Serous glands.
L.T.	Lymphoid tissue.
D.	Decussation.

Fig. 1.

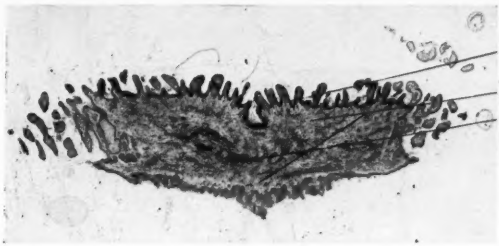
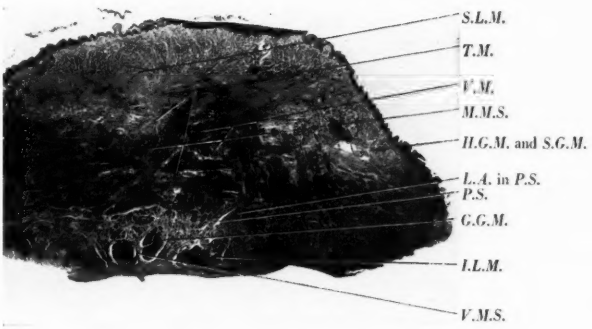


Fig. 2.

Fig. 3.



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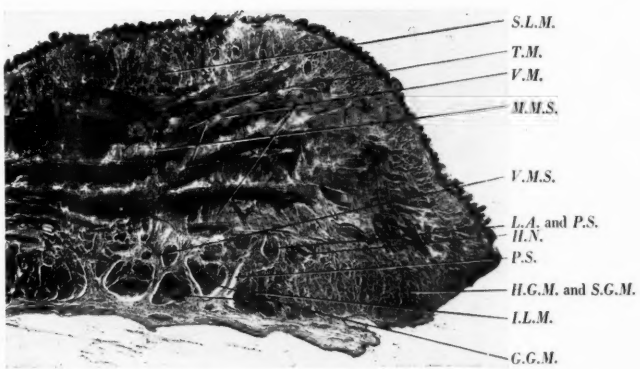

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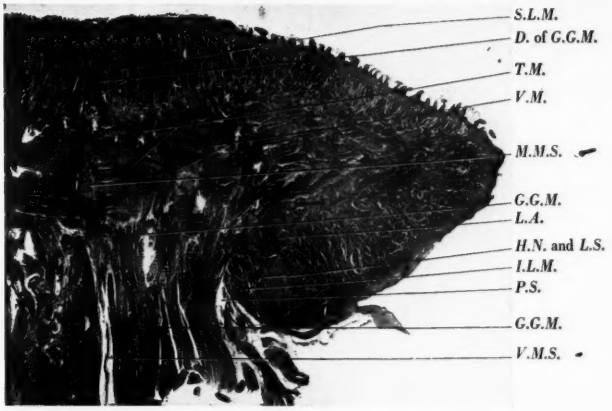

Fig. 5.

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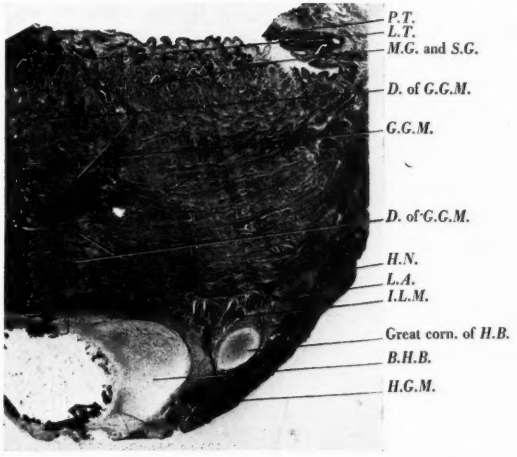

Fig. 7.

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A REVISION OF THE EXTENSOR MUSCLES OF THE FOREARM IN TETRAPODS

By R. WHEELER HAINES

Department of Anatomy, St Thomas's Hospital Medical School

INTRODUCTION

It is among the extensor muscles of the forearm that the most dramatic changes of nerve supply are to be found. In some animals, such as turtles, there are four motor nerves to the extensors; in others, such as frogs and crocodiles, there may be but one. But the changes have been little understood, and there is as yet no agreement as to the primitive state from which the modern structures have been derived. In the case of the muscles also, though they are better known than the nerves, the relationships in the various groups of animals are not understood, and the ancestral plan has never been clearly defined. In this paper, from a study of the most primitive types now available, the turtles, *Sphenodon* and the urodele amphibians, the primitive conditions are defined, and illustrated by a reconstruction of an early reptile, and then the arrangements in the more specialized types are considered. The literature, particularly the works of Brooks, Ribbing and Howell, will be considered in the text.

The material contains representatives of all living orders of reptiles and amphibians which possess limbs, with a monotreme and a marsupial to represent the mammals. Classical types have been used in preference to relatively unknown animals except where they appeared too highly specialized. I have to thank the trustees of the Thomas Smythe Hughes Fund for a grant towards the purchase of specimens, and the Conservators of the Museum of the Royal College of Surgeons, Prof. A. B. Appleton, Prof. Gowland and Prof. Watson for special material.

MUSCLES AND NERVES OF PRIMITIVE TYPES

Chelonians. The turtles, in spite of the development of the carapace, seem to have preserved a very primitive type of forelimb. *Emys* is a good type chosen by Ribbing (1907).

Muscles. From the humerus arise three groups of muscles, a humerodorsalis, an ulnar extensor mass, and a radial extensor series including a tractor radii. The humerodorsalis has eight slips of insertion, to the bases of the metacarpals from the ulnar side of I to the radial side of V. The main ulnar extensor passes to the shaft and lower end of the ulna and to the pisiform, and

an accessory ulnar extensor, peculiar to *Emys*, passes from the lower end of the ulna to the pisiform.

The four radial muscles are described by Ribbing (1907, extensor antibrachii et carpi radialis *a*, *b*, *c* and *d*), but have not been figured. They will be called here the extensor radialis superficialis, profundus and intermedius, and, to avoid the name extensor for a muscle supplied by a flexor nerve, the

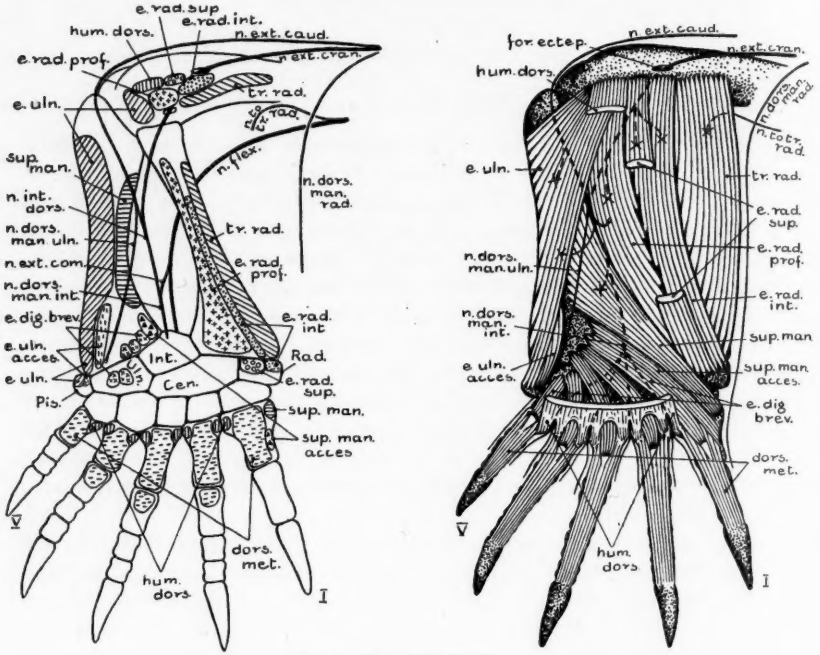

Fig. 1. *Emys blandingii*.

tractor radii. The superficialis inserts on the radiale, the others on the radius, except for a small slip of the intermedius to the radiale. The three extensors are supplied by the n. extensorius cranialis after it has pierced the ectepicondylar foramen, the tractor by a special flexor nerve which pierces the biceps-brachialis mass.

Of the shorter muscles the supinator manus passes from the shaft of the ulna to the base of metacarpal I, and an accessory supinator inserts on its shaft. The five extensores breves, three of which arise from the ulna, and two from the ulnare, pass between the insertions of the humerodorsalis to the digits, where they blend with the dorsometacarpales. These arise from the metacarpals, and in the middle three digits from the proximal phalanges, and insert on the bases of the distal phalanges.

Nerves. There are four motor nerves in the extensor region, the n. extensorius cranialis (Sieglbauer, 1909) which passes through the ectepicondylar foramen, the n. extensorius caudalis which lies in the groove between the ectepicondyle and the olecranon, the n. extensorius communicans which reaches the extensor region from the flexor nerves by passing through the radio-ulnar interosseous space, and the special nerve to the tractor radii. The cranialis and caudalis join to form the dorsal interosseous nerve, which is joined deep to the supinator manus by the n. communicans.

Of the three cutaneous nerves to the digits, the nn. dorsales manus ulnaris, intermedius and radialis, the ulnaris is formed from the n. extensorius caudalis just as it joins the cranialis, and passes superficial to the supinator manus and then along the groove between the ulnar extensors and the extensor brevis digiti V. The intermedius is the direct continuation of the dorsal interosseous, while the radialis is a special branch from the extensor nerve in the upper arm, and passes down the radial border of the forearm and hand. This account differs from that given by Ribbing (1907) in a few particulars. The radial and ulnar extensors are said to reach the metacarpals, the short extensors to arise from the intermedium, and the dorsometacarpals to reach the carpal bones. The communicating extensor nerve was not seen. The nerve to the tractor radii was first noticed later (1911*a*).

The muscles of other chelonians appear to resemble those of *Emys*, apart from peculiarities such as the accessory ulnar extensor and supinator manus (*Cyclanorbis*, *Chelhydra*, *Hydromedusa*, Sieglbauer, 1909; *Sternothaerus*, Ribbing, 1907; confirmed in *Chelhydra*, *Sternothaerus* and *Testudo*, R. W. H.). In some turtles with specialized swimming paddles (*Thalassochelys*, Sieglbauer, 1909; *Trionyx*, Ogushi, 1913) the muscles are modified, but seem to have been derived from an *Emys*-like state.

Sphenodon. In *Sphenodon* the humerodorsalis has only four insertions instead of eight, for those to the radial sides of the metacarpals have been lost. The extensor ulnaris has become subdivided into an extensor antibrachii ulnaris to the upper half of the ulna, and an extensor carpi ulnaris to the pisiform and base of the fifth metacarpal with an extension to the humerodorsalis. The radial muscles are arranged as in *Emys*, but only the superficialis reaches the radiale. The supinator manus and a small slip of the extensor brevis digiti I arise from the ulna, the rest of the extensores breves from the intermedium and ulnare. The dorsometacarpals are much more complicated than in chelonians. Those for digits II, III and IV arise each by three heads, from their own metacarpals and from those on either side, the first arises from its own metacarpal and from II and the more radial centrale, and the fifth from its own metacarpal only.

The four motor nerves described in chelonians are found again in *Sphenodon*, but the n. dorsalis manus ulnaris now arises on the flexor surface and winds round the ulnar border of the hand to the ulnar side of the fifth digit.

The muscles and nerves of *Sphenodon* have been described by Brooks (1889),

Osawa (1898), Ribbing (1911) and Miner (1925). The account given here agrees best with Ribbing's text, Miner's figures of the muscles, and Brooks's of the nerves.

Salamandra. *Salamandra* has been chosen as a type of the Amphibia, rather than the better known *Megalobatrachus* or *Necturus*, as its muscles seem on the whole more primitive. The muscles have been well illustrated by Perrin

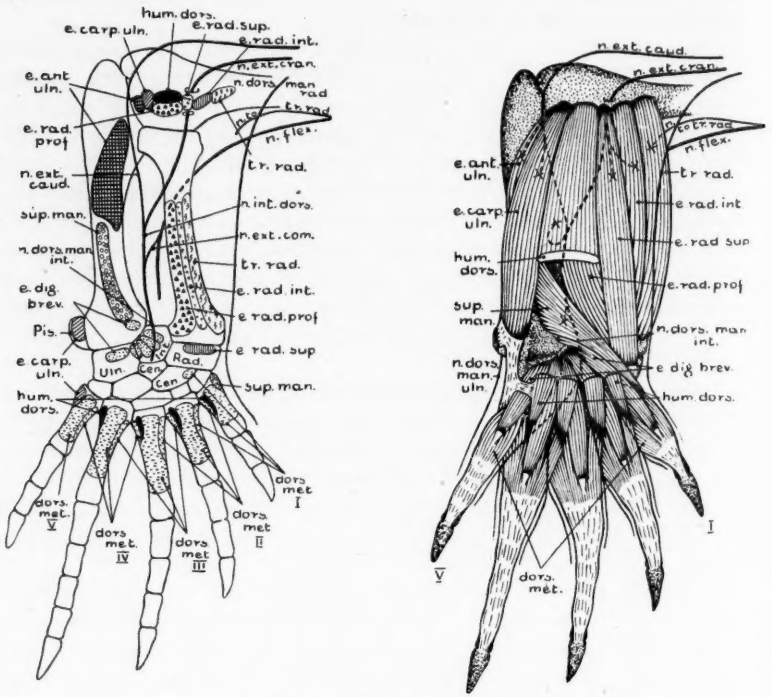

Fig. 2. *Sphenodon punctatum.* Skeleton from Miner (1925).

(1899), with their bony attachments, and Sieglbauer's (1904) illustrations of the nerves are excellent. For this paper the arrangements have been checked by a complete serial section through the forearm and hand.

The humerodorsalis is inserted as in chelonians by two slips to the base of each metacarpal, but that to the first is usually missing, and on the ulnar side the fifth digit has been lost (Gregory *et al.* 1923) and with it the attachment to the metacarpal, so that only six slips remain. The extensor carpi ulnaris, with the loss of the pisiform, inserts on the intermedio-ulnare, and a powerful extensor antibrachii ulnaris passes to the ulna.

There are only three muscles in the radial group, all supplied by extensor nerves, two by twigs which spring from the n. dorsalis manus radialis before it

pierces the most radial of the muscles. The identification of the extensor radialis superficialis and profundus is clear, both from their attachments and relations, and from the courses of the nerves, for the supply of the superficialis reaches it by passing on the radial side of the profundus, so separating it from the rest of the musculature. The most radial muscle is probably the intermedius, the tractor radii having disappeared together with its nerve supply. It might on the other hand be a fused intermedius and tractor supplied only by an extensor nerve, but this is less probable. Since there must be some un-

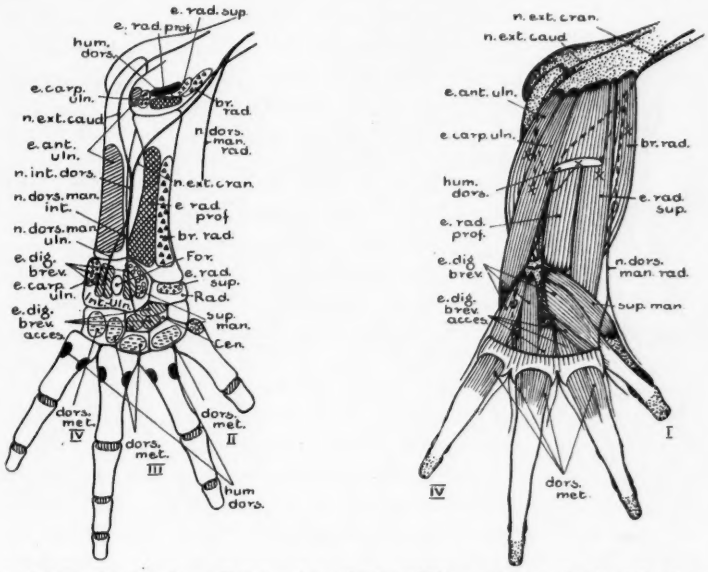

Fig. 3. *Salamandra maculosa.* Skeleton from Perrin (1899), muscles partly from Perrin, nerves partly from Sieglbauer (1904), checked by a complete serial section.

certainty, and since the matter has never before been discussed in the light of the four reptilian muscles and their nerve supplies, it is convenient to call the muscle the brachioradialis, as all authors are agreed that it corresponds to that muscle in mammals.

The supinator manus is peculiar in its origin from the carpus only. Ribbing (1907), supposing that the first digit of urodeles was equivalent to the second of reptiles, could not consider the supinator manus homologous in the two groups. He postulated an ancestral form in which all the short muscles inserted on the terminal phalanges, and later an independent transference of the supinator to the marginal metacarpals, a hypothesis which is no longer necessary. The short extensors to digits I, II and IV take origin from the intermedio-ulnare, and in the specimens figured by Perrin and Sieglbauer a slip also passed to digit II. A second group of origins, probably migrated parts of the short extensors,

arise from the centrale. The dorsometacarpals pass from the carpals to digits II, III and IV.

The cranial extensor nerve pierces the profundus, instead of passing deep to it. The communicating extensor nerve is absent in *Salamandra*, but is found in several urodeles. The n. dorsalis manus ulnaris is, as in *Emys*, derived from the extensor nerves, and supplies the third cleft as well as the ulnar border of the hand, and the n. dorsalis manus radialis, after piercing the brachioradialis, supplies the first cleft.

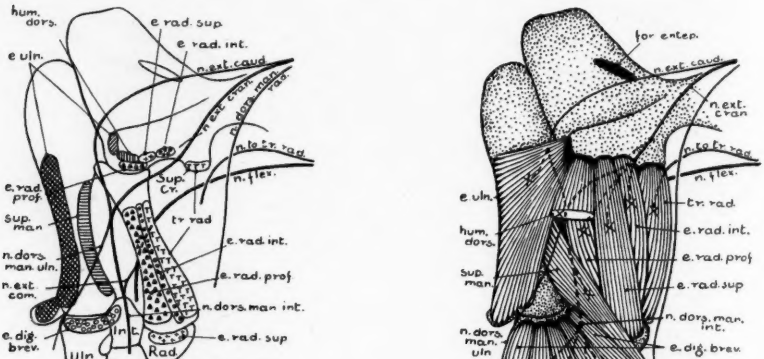

Fig. 4. *Ophiacodon mirus*, a Permian reptile. Skeleton from Williston & Case (1913).

This description agrees with those of Eisler (1895), Perrin (1899) and Sieglbauer (1904) except in some unimportant details. Other unspecialized urodeles agree in general plan (*Triton, Necturus, Ambystoma*, Sieglbauer and Ribbing; *Necturus*, Wilder, 1912). In *Necturus* all the short extensors seem to have disappeared, leaving only the dorsometacarpals on the back of the manus. The extensor ulnaris is usually undivided, *Salamandra* and *Triton* having probably developed the division into an antibrachial and a carpal part independently of the reptiles such as *Sphenodon*.

Ophiacodon. The primitive form of tetrapod limb can now be defined in some detail, and can be reconstructed in *Ophiacodon*, an early reptile described by Williston & Case (1913), though the individual peculiarities cannot of course

be deduced. The humerodorsalis arises from the ectepicondyle, and is inserted by eight slips to the bases of the metacarpals as in *Emys*. A single undivided extensor ulnaris passes as in *Emys* from the distal end of the ectepicondyle to the shaft and lower end of the ulna and to the pisiform, but probably not to the fifth metacarpal. The four radial muscles are all perfectly distinct, the extensor radialis superficialis passing as usual to the radiale, and the profundus and tractor radii to the radius. Probably the intermedius also is confined to the radius, as the extension below the wrist in *Emys* is unusual in reptiles. The origins of this group are more difficult to define.

As noted by Ribbing (1911*a*), those living animals which have an ectepicondylar foramen have also a special motor nerve from the flexor region, the nerve to the tractor radii, while the animals which have no foramen (amphibians, crocodiles and mammals) have no such nerve (exception in tejid lizards, R. W. H.). If the nerve, as suggested here, always supplies the tractor radii and no other muscle, it may be taken that the presence of an ectepicondylar foramen is associated with an arrangement of the radial muscles similar to that in *Emys* and *Sphenodon*. Now though the foramen is found in several fossil groups, it is primitively absent in both reptiles and amphibians (Romer, 1922), its place being taken by a deep groove between the ectepicondyle and the "supinator crest". So the cranial extensor nerve lying in the groove runs between the musculature arising from the two bony processes. In later reptiles in which these two processes have become fused to close over the foramen, the origins of the musculatures are contiguous, so that it is no longer possible to say with certainty which muscles arose from the one process and which from the other. Since, however, the tractor is separated from the other muscles by its nerve supply it seems probable that it alone arose from the crest, and the rest from the epicondyle as shown in the reconstruction. Again the early amphibians possessed a crest similar to that of *Ophiacodon*, and the tractor was presumably well developed. There is no evidence that the crest ever joined the epicondyle as in reptiles; it seems rather to have disappeared together with the muscle and its nerve supply, leaving the cranial extensor nerve proximal to the remaining muscles, as would be expected if they arose originally from the epicondyle.

The supinator manus and short extensors are always closely related in both reptiles and amphibians, and in *Ophiacodon* probably stretched from the ulna to the intermedium and ulnare, for the arrangement in *Salamandra*, where they are confined to the hand, is peculiar. The dorsometacarpals are more difficult to reconstruct as they differ widely in *Emys*, *Sphenodon* and *Salamandra*. It seems perhaps most probable that the single metacarpal origin in *Emys* is primitive, and that in *Sphenodon* and lizards, and to a lesser degree in anurans, they have spread on to the neighbouring metacarpals, while in urodeles they have moved proximally on to the carpal bones.

The motor nerves of primitive tetrapods probably resembled those of *Sphenodon*, with all four possible routes developed, and it seems safe to attempt

their reconstruction, although in other regions it has been usual to show the muscles only in reconstructions. The three digital nerves are arranged on the chelonian plan, with the n. dorsalis manus ulnaris springing from the extensor nerves and not from a flexor nerve as in *Sphenodon*. The only doubtful point is the presence of a communicating extensor nerve. This is found in most urodeles, in chelonians and in *Sphenodon* and lizards, and if not ancestral, must have been developed independently in these three lines. It seems more probable that it has been lost in a few urodeles, and in anurans, crocodiles and mammals, all relatively specialized animals.

The structure of the primitive forearm has not been precisely defined by other workers, so that no exact comparisons are possible. Ribbing (1907) and Miner (1925) consider the metacarpal insertion of the humerodorsalis secondary and its insertion on the terminal phalanges primitive, but in all reptiles and most urodeles the insertions are on the metacarpals, and Howell (1936*a*) considers these primitive. The four radial muscles are recognized by Ribbing (1907 *Emys*, 1911 *Sphenodon*), but not the constant relations to their nerve supplies, nor the loss of the tractor in urodeles, nor is the primitive condition defined. Howell, working on *Necturus* in which the tractor is missing, and the lizard Iguana in which the superficialis is missing, and not recognizing the double nerve supply, describes only two muscles, an extensor carpi radialis longus and brevis. The interpretation of the nerves agrees in the main with Ribbing (1908) but other authors have been inclined to regard the communicating nerve as an anomalous structure rather than an ancestral heritage, for its wide distribution in reptiles and amphibians has not been appreciated.

The morphology of the tractor radii is doubtful, for its nerve supply suggests that it is a flexor though associated with extensor muscles. There is no absolute objection to the origin of a flexor muscle from the radial side of the humerus. In the hind limb the tibial head of the gastrocnemius arises on the tibial side of the main mass of the flexor musculature.

It can now be seen that the turtles come very near to the primitive type, the chief differences being those leading to the formation of an ectepicondylar foramen. *Sphenodon* is specialized in the splitting of the extensor ulnaris and its extension to the fifth metacarpal, the reduction of the insertions of the humerodorsalis, and the new origin of the n. manus ulnaris, while all urodeles have lost the bones and muscles of the ulnar border of the hand, and also the tractor radii with its nerve supply.

AMPHIBIAN TYPES

Megalobatrachus. *Megalobatrachus* and the related *Cryptobranchus* are specialized, secondarily aquatic forms, but have unfortunately been chosen for intensive study by several anatomists (Humphrey, 1872; Osawa, 1898; Miner, 1925, etc.), probably on account of their large size, and have had a profound effect on myological theory. The extensor ulnaris is undivided. The three radial extensors are arranged as in *Salamandra*, the tractor radii being lost as in all

other urodeles. The supinator manus takes origin from the ulna instead of being confined to the hand. The n. dorsalis manus ulnaris has encroached on the intermedius as in *Salamandra*, but not the radialis. The communicating extensor nerve is well developed.

So far *Megalobatrachus*, where it differs from *Salamandra*, is the more primitive, but the muscles to the digits are profoundly modified. Apart from a single insertion into the base of the fourth metacarpal the humerodorsalis joins the short extensors and reaches the terminal phalanges. The dorsometacarpals have disappeared completely, the supposed remnant described by Miner being

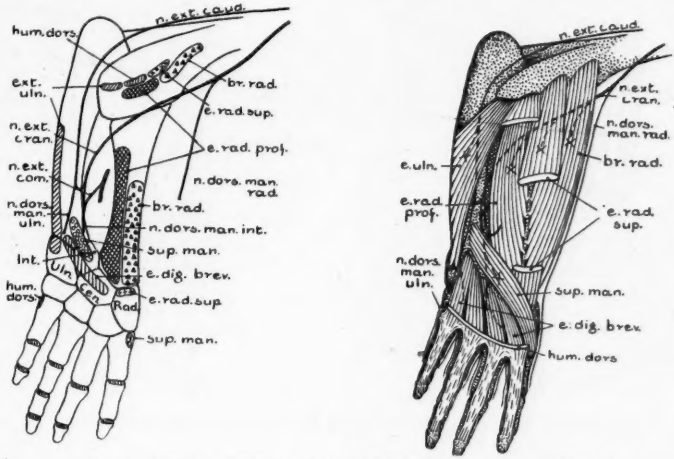

Fig. 5. *Megalobatrachus japonicus*. Skeleton from Miner (1925).

a flexor muscle supplied by a flexor nerve. It is clear then that this arrangement cannot be considered primitive. Other supposed extensions of the humerodorsalis beyond the metacarpals seem to be erroneous (Brooks (1889) in *Necturus*, corrected by Wilder (1912) and Howell (1936*a*), Ribbing (1907) in *Ambystoma*, absent according to Sieglbauer (1904), confirmed R. W. H.; Eisler (1895) in *Salamandra*, corrected by Perrin (1899)).

Eryops. The muscles of *Eryops*, a large land-living Permian form, have been carefully reconstructed by Miner (1925), and this is the only detailed reconstruction of the forearm and hand so far attempted in any animal. It is now possible to correct this work in several details, and to add the courses of the nerve trunks.

The humerodorsalis is shown inserted on the bases of the metacarpals, not on the phalanges as in *Megalobatrachus*, which was one of Miner's types. The extensor ulnaris is shown single, and inserted below the wrist only on the pisiform and ulnare, below which it does not extend in urodeles and chelonians, not inserted more distally as in *Sphenodon*, Miner's other type. The similarity of the

lower end of the humerus to that of *Ophiacodon* justifies the reconstruction of four muscles as in reptiles, not three as in modern urodeles, and apart from the superficialis all are inserted on the radius. Miner figures these muscles correctly in *Megalobatrachus* and *Sphenodon* apart from the separation of the tractor and intermedius, but in his description of *Sphenodon* and his reconstruction of *Eryops* the profundus is supposed to insert below the wrist. The nerves are shown as similar to those of *Ophiacodon*.

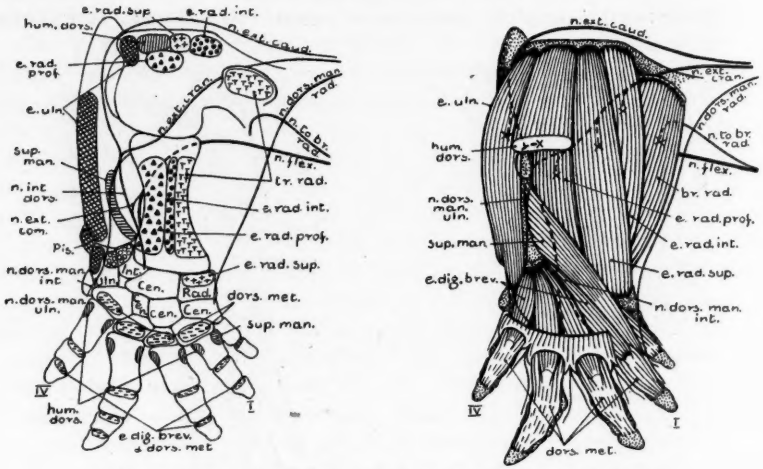

Fig. 6. *Eryops megacephalus*. Skeleton from Gregory *et al.* (1923).

Rana. In anuran amphibians the musculature is very highly specialized. In *Rana catesbiana*, the giant American bullfrog, the extensores carpi and antibrachii ulnaris are separate, and the humerodorsalis reaches the terminal phalanges of digits II, III and IV, with a small insertion on metacarpal IV. The extensor radialis profundus (Gaupp, 1896, "Flexor antibrachii lateralis profundus") is arranged as usual. The brachioradialis (Gaupp, "F. antib. lat. superficialis, cap. inferius") can be recognized by its position, although its relation to the cranial extensor nerve has changed. The extensor radialis superficialis (Gaupp, "E. carpi radialis, cap. inferius") also has the usual relations to the nerves, but is inserted on the peculiar ligamentous arch which stretches from the radio-ulna to the intermedius. Two small accessory slips (Gaupp, "e. carpi radialis, cap. superius and flexor ant. lat. superfic., cap. superius"), probably migrated parts of the extensor radialis superficialis or intermedius, or both, take origin above the nerve, and are inserted the one with the extensor radialis superficialis and the other directly on the radio-ulna. This group of muscles, which varies in development in the two sexes (Gaupp, 1896; Howell, 1935), needs further study.

The supinator manus is large, and has an unusual origin from the humerus

besides its origin in the forearm. Between the two heads passes the nerve to the humerodorsalis and the n. dorsalis manus ulnaris, and these nerves must have been crossed by the humeral head of origin in its migration to the humerus. Ribbing (1907) thought that this head was a part of the humerodorsalis which joined the supinator, but this seems improbable on account of its insertion on the radial side of metacarpal I, and on account of its deep position. The short extensors again are specialized. One group, the extensores breves superficiales,

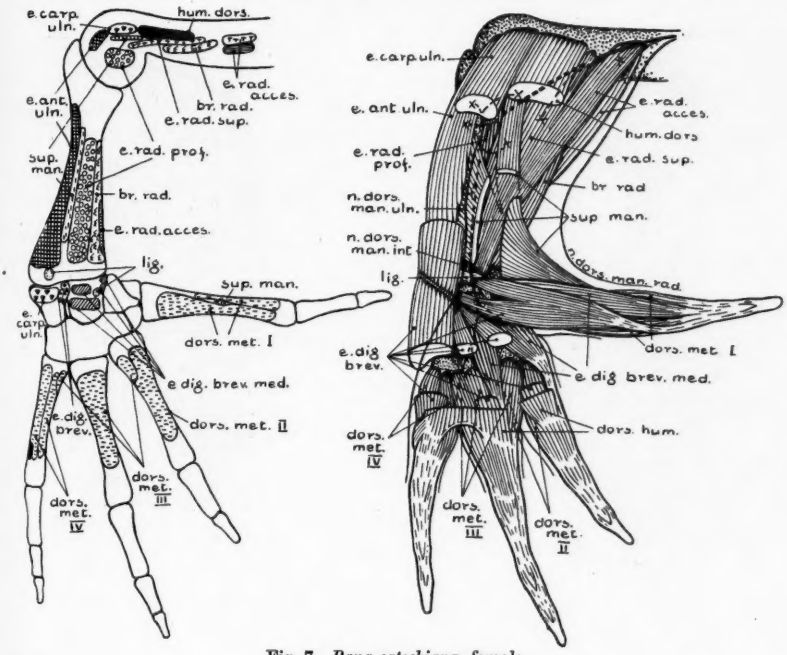

Fig. 7. *Rana catesbiana*, female.

has a slip to I from the tendinous arch and ulnare, to II and III from the ulnare, and IV from the extensor carpi ulnaris insertion, while a deeper group, the extensores breves medii to I, II and III, arises from the radiale, that to II by two heads which embrace the attachment of the tendinous arch. The dorsometacarpales are highly developed, that to III arising from metacarpals II, III and IV, as the corresponding muscle in *Sphenodon*.

This description agrees with those of Gaupp (1896, *Rana esculenta*) and others. Other anurans seem similar (Ribbing, 1907, 1911*b*, literature), though the lack of adequate illustrations makes comparisons difficult. It is clear that all frogs are highly specialized when compared with other groups, and that even if some of the muscles have been identified wrongly, there must have been some changes in the relations of the muscles to the nerves.

SPECIALIZED REPTILIAN TYPES

Varanus. The most satisfactory type of lizard known is *Varanus*, for though highly specialized in other regions of its body, its forearm is relatively primitive, and at the same time is large enough to allow accurate dissection. It is best to compare the lizards, not directly with the primitive reptiles, but with *Sphenodon*, for the specializations found in *Sphenodon* reappear in the lizards, together with several specializations of their own. It is highly probable

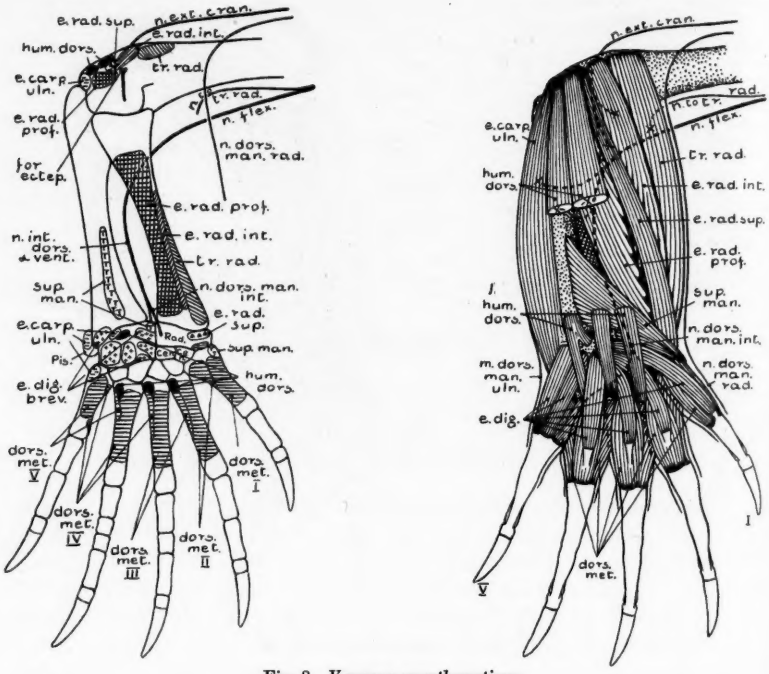

Fig. 8. *Varanus exanthematicus.*

that the two lines are closely related (Broom, 1925), and certainly the ancestors of the lizards must have resembled *Sphenodon* very closely as regards their limbs.

The humerodorsalis has two parts, one to metacarpals II and III, the other to IV and the ulnare. The slip to I has been lost. The extensor carpi ulnaris reaches metacarpal V as in *Sphenodon*, with slips to the pisiform and ulnare. The extensor antibrachii ulnaris, already reduced in *Sphenodon*, has been lost. The radial group is said by Ribbing (1907) to consist of two muscles only, the superficialis and a combined mass representing the rest. In *Varanus exanthematicus*, *griseus* and *niloticus* however, all the muscles described in *Sphenodon* are perfectly distinct. The supinator manus arises from the ulna

with a small slip from the ulnare, and the short extensors from the ulnare except an extra slip to V from the lower end of the ulna. The dorsometacarpals are arranged as in *Sphenodon*, but the ulnar slips of digits I, II and III have been lost, while that to IV retains its slip from metacarpal IV, but this now arises indirectly from a tendinous arch.

The nerves are highly specialized in lizards. The tractor radii is served as usual by a special nerve, and the three radial extensors by the n. extensorius cranialis, which however ends in these muscles. The n. extensorius caudalis is

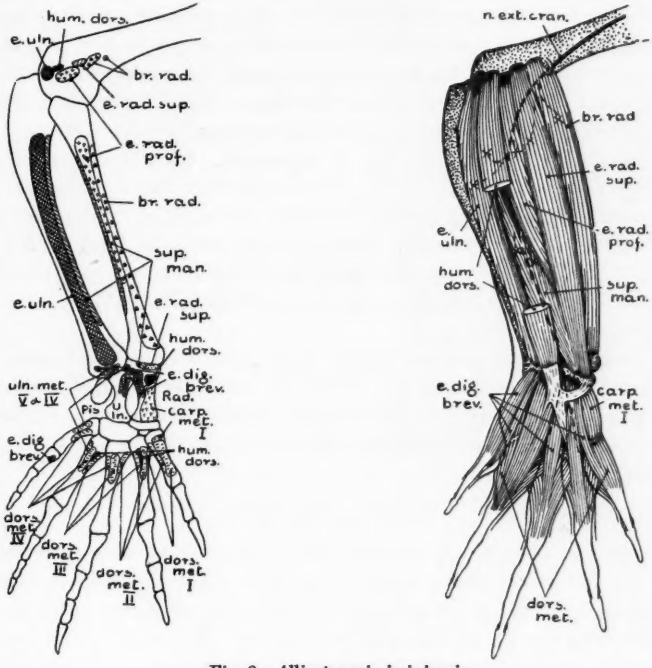

Fig. 9. *Alligator mississipiensis.*

absent, and all the rest of the muscles are supplied by a greatly enlarged communicating nerve, which also forms the sole source of the dorsal interosseous nerve and the n. dorsalis manus intermedius.

The muscles of *Iguana*, the type chosen by Rabl (1916) and Howell (1936*a*) to represent the reptilian structure, are similar to those of *Varanus*, except for the loss of the superficialis, which often occurs in lizards (Ribbing, 1907). The tejid lizards are of interest as they have lost the ectepicondylar foramen, so that the n. extensorius cranialis has to pass among the origins of the muscles instead of through the bone. In *Ameiva* the extensor radialis superficialis has been lost, so that, as in *Iguana*, the nerve supplies only two muscles, the intermedius

and profundus. Now if the reconstruction of *Ophiacodon* is correct, the nerve when set free of the bone, if it lay in its old position, would be expected to reach the profundus by passing between the tractor and the intermedius. This is in fact the case, giving strong confirmation for the reconstruction. In other animals the loss of the foramen combined with the retention of the tractor is unknown.

Crocodilians. The crocodiles and alligators are a highly specialized group. In the alligator the humerodorsalis is inserted chiefly into metacarpal II with extensions to IV and the radiale, in Ribbing's *Crocodilus* into II and III. The extensor ulnaris is confined to the ulna. The three radial muscles are probably equivalent to those of urodeles, the tractor radii having been lost. The brachioradialis arises partly from the humerus, and partly from a fibro-tendinous arch over part of the flexor musculature of the upper arm. The supinator manus is enormously developed, and its origin has spread from the ulna on to the radius, an extension found elsewhere only in mammals. Its insertion on the first metacarpal has been lost, and a new one formed on the radiale in common with the extensor radialis superficialis. Probably the radiale metacarpale I is a distal fragment of this muscle. These changes are associated with a great mobility of the wrist joint, and the use of the elongated carpus as an extra limb segment. The short extensors and dorsometacarpals are arranged as in *Sphenodon.*

The crocodiles and anurans are the only animals known to have retained but one motor extensor nerve, a n. extensorius cranialis. The n. dorsalis manus ulnaris arises as in lizards and *Sphenodon* from the flexor nerves, but here has spread to supply the fourth cleft as well as the ulnar border of the hand, as in mammals.

MAMMALIAN TYPES

Monotremes. The muscles of mammals are difficult to interpret in reptilian terms, but the monotremes, though they have many specializations of their own, appear to be relatively primitive in the extensor region of the forearm.

In *Echidna* the humerodorsalis has become the extensor digitorum communis by changing its insertion from the metacarpals to the terminal phalanges, with a slip to the basal phalanx of the pollex. This has probably occurred as in frogs and cryptobranchs by a secondary fusion with the tendons of the short extensors. Howell (1936) suggested that the tendons loosed themselves from the metacarpals and laid hold of the neighbouring fascia so as to modify it into a series of tendons, but it seems doubtful whether new tendons are ever formed in this way. It is more likely that a single embryonic matrix is fully subdivided in reptiles forming the humerodorsalis and short extensors, and that in mammals its remains undivided distally, so that both muscles are continued to the digits.

The extensor carpi ulnaris has, besides its old origin from the humerus, a new one from the ulna, and its insertion reaches the terminal phalanx, a

monotreme specialization. The extensor digitorum ulnaris lies between it and the humerodorsalis. It has no clear homologue in reptiles, but seems to be derived from a radial migration of a part of the extensor carpi ulnaris. Ribbing (1907) and Howell (1936) mention this derivation, but set it aside in favour of an origin from the communis, Ribbing on the grounds that in *Echidna* it has blended with this muscle in the forearm, a condition not seen in the specimen figured. In fact in *Echidna*, where the three digital extensors co-exist, the ulnaris is the deepest of all, separated from the communis by the pro-

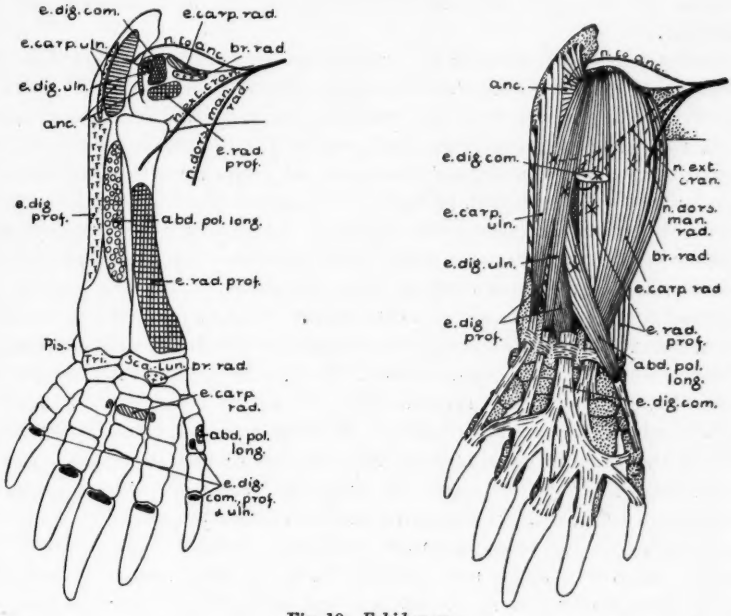

Fig. 10. *Echidna* sp.

fundus, and it is difficult to imagine the migration of the ulnaris through or round the border of this muscle. Brooks (1889) and Eisler (1895) consider the ulnaris a part of the reptilian extensores digitorum breves which has migrated from the ulna to the humerus, but this is improbable, for if any fibres should migrate they would be the uppermost, that is the fibres of the radial digits, not the ulnar ones. The question is discussed in detail by Straus (1938), with whose opinions the present author is in agreement. The extensor digitorum profundus, formed from the coalescence of the originally separate bellies of the extensores breves of reptiles, though blended with the communis, can still be traced to all digits.

The anconeus, well developed in monotremes, has usually been considered

as part of the triceps which has migrated distally carrying its nerve supply with it, partly on account of its origin, which may be from the shaft of the humerus in some animals, and partly on account of its peculiar nerve supply, which reaches it by passing between the epicondyle and the olecranon. Brooks (1889) however, from a comparison with the conditions in *Sphenodon*, identified the muscle as an extensor antibrachii ulnaris, and the nerve as a n. extensorius caudalis, and this was accepted by Ribbing (1907), but rejected by Howell (1936*a*) on account of the nerve supply. Conditions in *Echidna* support Brooks's theory, though the reduction from the primitively wide ulnar insertion of the extensor ulnaris must have occurred independently in *Sphenodon* and in mammals.

Of the four radial muscles the tractor has disappeared, though it is just possible that it is represented by Westling's (1889) "brachioradialis" which is supplied by a flexor nerve. The extensor carpi radialis is the old superficialis which has migrated to the metacarpals. The profundus has become the supinator, which in monotremes retains its old origin from the humerus and wide insertion on the radius, as well as its position superficial to the cranial extensor nerve, and between the nerves to the extensor carpi radialis and extensor digitorum communis. Some such derivation has been accepted by all authors except Howell (1936*a*), who thought it might be a part of the supinator manus. This, however, would involve changes in its origin, its insertion and its relation to the nerve to the humerodorsalis, besides the suppression of the profundus, and appears unlikely. The brachioradialis is inserted on the scapho-lunar, a monotreme specialization, also found in marsupials, and as in urodeles appears to be an intermedius. Ribbing derived the brachioradialis as well as the extensor carpi radialis from the superficialis of reptiles, chiefly on account of its insertion below the wrist. But the radial insertion appears primitive in mammals. The supinator manus or abductor pollicis is arranged as in reptiles. The extensor digitorum profundus, formed from the short extensors, arises as a single mass entirely from the ulna, instead of partly or wholly from the carpus as in reptiles, and after fusion with the extensor communis appears to be distributed to all five digits. Dorsometacarpals are not developed.

Of the four motor nerves only two remain in mammals, the n. to anconeus or n. extensorius caudalis, and the motor part of the radial n. or n. extensorius cranialis which supplies all the other muscles. The n. dorsalis manus radialis has become peculiarly enlarged in monotremes and supplies all the digits (Westling, 1889).

Ornithorhynchus is similar in structure (Howell, 1936*b*, literature). The extensor digitorum ulnaris is not so differentiated from the extensor carpi ulnaris as in *Echidna*, and still occupies the same osseo-fibrous tunnel on the back of the ulna. Howell states that the anconeus is innervated in common with the extensor carpi ulnaris. His "extensor carpi radialis longus" is the extensor carpi radialis, undivided in *Ornithorhynchus* as in *Echidna* (Eisler,

1895), his "e.c.r. brevis" is the brachioradialis, and his "brachioradialis" the supinator, and all are arranged as in *Echidna.*

Didelphys. In *Didelphys,* the type chosen by Romer (1922) and others, the extensor digitorum communis is inserted into only four digits, so emancipating the first for special movements, and the tendons are connected with each other by expansions over the metacarpals. The extensor carpi ulnaris has the normal mammalian insertion on the fifth metacarpal as in lizards, and the anconeus is arranged as in *Echidna.* The extensor digitorum ulnaris splits into two tendons, one to digit V only, the other to III, IV and V. The extensor

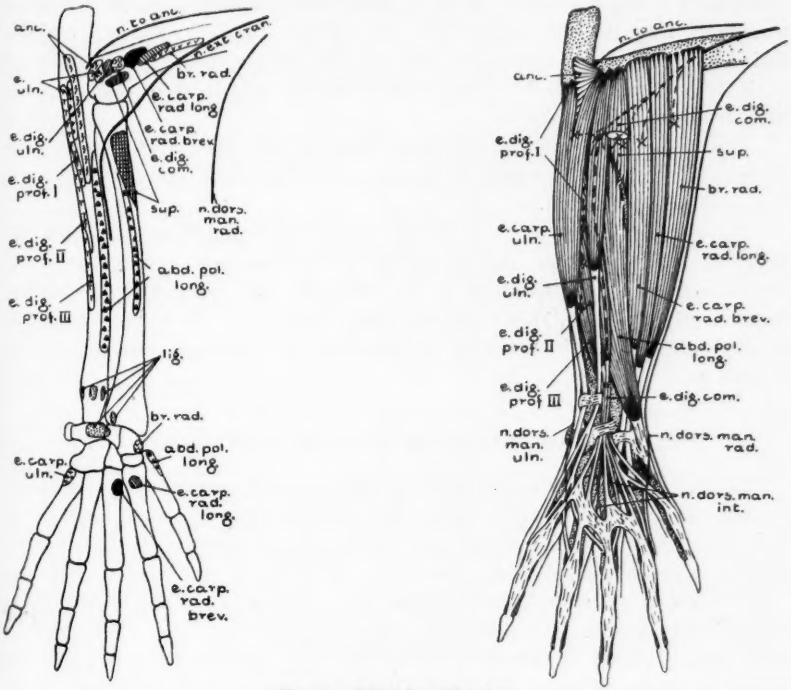

Fig. 11. *Didelphys virginiana.*

carpi radialis is now split as in most mammals into a longus and brevis, passing to metacarpals II and III. The brachioradialis has a peculiar insertion, chiefly into the scaphoid, with a radial expansion to the abductor pollicis longus and an ulnar to the lunate and triquetrum. The supinator is now reduced as in most mammals, so as to reach only the upper end of the radius. The abductor pollicis is large and now arises from both radius and ulna. The three bellies of the deep extensor are inserted into digits I, I and II, and III, the last joining the tendon of the extensor ulnaris to III, but these insertions are variable in *Didelphys* (Coues, 1872; Straus, 1938).

There is no mammal now living that shows the primitive state of the short extensors, but in the common ancestors of the monotremes and other mammals they must have been similar to those of reptiles, with five separate slips to the digits. For in *Echidna* the common belly supplies all the digits, and in other mammals, though the number of slips has been reduced to four as in *Tarsius* (Ribbing, 1907), three as in *Didelphys*, or two as in man, those that remain are separate bellies. Brooks (1889) has described clearly the proximal migration of the short extensors of reptiles on to the forearm of mammals, and this has been accepted by later authors. Ribbing (1907), however, believed that they were primitively a single muscle mass as in monotremes, and that when, in typical mammals, they became independent, they inserted only on digits I and II. This however is not in agreement with the state of the muscles in reptiles, or with their insertion into five digits in monotremes, and three or four digits in most primitive mammals.

The motor nerves are arranged as in *Echidna*, but the cutaneous nerves are more primitive, though variable (Straus, 1938), the three nn. dorsale manus being all well developed.

This account agrees well with that of Coues (1872) and *Didelphys* appears a good type of the mammals, though it may not be primitive in the insertion of the brachioradialis or the splitting of the extensor carpi radialis, very variable features (MacCormick, 1886; Ribbing, 1907, literature). The changes found in other mammals and in primates in particular are discussed fully by Straus (1938).

DISCUSSION OF SPECIAL POINTS

Nerves. The muscles of the radial group are quite constant in their innervation, the tractor radii by a special nerve, and the rest by the n. extensorius cranialis. All the other muscles are variable, for all may be supplied by the n. extensorius cranialis (frogs, crocodiles), or by the n. extensorius communicans (lizards), or they may be supplied by a combination of these and the n. extensorius caudalis (the primitive tetrapod condition), or by the cranialis and caudalis alone (*Salamandra*, mammals). The caudalis when present may supply only the extensor antibrachii ulnaris (mammals), or the whole of the ulnaris and part of the humerodorsalis (primitive state). Clearly in this region little reliance could be placed on the nerve supplies if the homologies of the muscles were doubtful.

On the other hand though in other regions the courses of the motor nerve trunks are known to change (Haines, 1935), in the forearm they are remarkably constant. The n. extensorius cranialis always passes between the tractor and intermedius, and then deep to the intermedius and superficialis and deep to or through profundus. The n. to superficialis always separates the intermedius and profundus, and the n. to humerodorsalis the profundus and extensor ulnaris, whether it comes from the cranial extensor n. or the communicating n.

Again the n. extensorius caudalis always passes deep to the extensor ulnaris or its derivatives and the dorsal interosseous deep to the supinator manus. Only in anurans are any variations found.

The cutaneous trunks are less constant, and in the hand they vary in their relations to the short extensors and dorsometacarpals. The n. dorsalis manus radialis may pass superficial to the brachioradialis (its primitive position), or through it (*Salamandra*), or deep to it (man).

Muscle functions. The functions of the muscles and the mechanical effects of their migrations cannot yet be determined in detail, for the movements of the joints are not sufficiently understood. Watson (1917) has shown however that in early tetrapods the movements of the humerus at the shoulder joint were limited to an antero-posterior swing of the horizontally placed humerus through a short arc, with little dorso-ventral movement and no rotation. The forearm, set at about a right angle on the distal end of the humerus (semiflexion in human terminology), passed downwards and forwards to meet the ground at an angle of 30–40°. Now a mammal in this position would, if it raised its hand from the ground, either raise the humerus at the shoulder joint, or rotate it so as to lift the forearm into a more horizontal position, but both these movements were impossible in early tetrapods. So the forearm at the elbow joint, besides flexion and extension, was capable of a considerable degree of elevation and depression in the sagittal plane. Pronation and supination were, if present, very limited, but the same effective movement was allowed by independent elevation and depression of the radius and ulna, and by movements of the wrist and carpo-metacarpal joints.

Now flexion and extension were chiefly carried out by the muscles of the upper arm, helped by those of the forearm which crossed the elbow joint, particularly flexion, used for dragging the animal forward, by the tractor radii. The other muscles inserting on the forearm, including on the extensor surface the major part of the ulnaris, and the radialis profundus and intermedius, were concerned in controlling elevation and depression, and the large epicondyles of early tetrapods were developed in association with these movements. The chelonians, having preserved a relatively primitive type of gait, in spite of the more anterior position of the elbow enforced by the carapace (Watson, 1914), still possess a very primitive musculature.

In *Sphenodon* and lizards, Fürbringer (1886) mentions besides flexion and extension only movements of the radius and ulna relative to one another. But here again elevation and depression appear important movements, although the shoulder joint is less rigid than in early reptiles. One of the muscles previously concerned mainly with elevation, the extensor ulnaris, is now partly or wholly concerned in wrist movements, while the others remain as before. Howell (1936*a*) after examination of *Necturus* and *Iguana* came to the conclusion that the muscles passing from the lower end of the humerus to the radius and ulna could do nothing but fix the elbow joint. But the muscles are well developed and fleshy, whereas if they could never contract they would be

ligamentous, and it seems more likely that they are active in the special movements discussed.

In mammals elevation and depression of the forearm have been lost, and pronation and supination acquired. The anconeus, a remnant of the ulnar part of the extensor ulnaris, is now small and acts as an extensor only, while the remainder of the muscle acts on the hand. The profundus in eutherian mammals is reduced, and specialized for its new function of supination. The tractor radii has gone, while the intermedius, though retaining its insertion on the radius, has changed its function, migrating up the shaft of the humerus and becoming a flexor, the brachioradialis. In monotremes, where as shown by Howell (1937) the bones at the elbow joint form a modified ball and socket articulation allowing flexion, elevation and rotation of the forearm, the profundus retains the reptilian type of insertion.

Too little is known of movements at the wrist for profitable discussion, and the reasons for the extension of the humerodorsalis to the terminal phalanges are not understood.

Table of homologies

Primitive tetrapods and turtles	*Sphenodon*	*Urodeles*	*Didelphys*
Humerodorsalis	Humerodorsalis	Humerodorsalis	Extensor digitorum communis
Extensor ulnaris	Extensor carpi ulnaris Extensor antibrachii ulnaris	Extensor ulnaris	Extensor carpi ulnaris Extensor digitorum ulnaris Anconeus
Tractor radii	Tractor radii	—	—
Extensor radialis superficialis	Extensor radialis superficialis	Extensor radialis superficialis	Extensor carpi radialis longus and brevis
Extensor radialis intermedius	Extensor radialis intermedius	Brachioradialis	Brachioradialis
Extensor radialis profundus	Extensor radialis profundus	Extensor radialis profundus	Supinator
Supinator manus	Supinator manus	Supinator manus	Abductor pollicis longus
Extensores digitorum breves	Extensores digitorum breves	Extensores digitorum breves	Extensor digitorum profundus
Dorsometacarpales	Dorsometacarpales	Dorsometacarpales	—

SUMMARY

1. The muscles of examples of all orders of reptiles and amphibians with limbs are described with their bony attachments, nerve supplies and relations to nerve trunks.

2. In primitive tetrapods the muscles consisted of an extensor ulnaris to the ulna and pisiform, a humerodorsalis to the metacarpals, a tractor radii and extensores radiales superficialis, profundus and intermedius, a supinator manus, extensores digitorum breves and dorsometacarpales. The tractor was supplied by a special nerve from the flexor region, the rest by the n. extensorius cranialis and caudalis, and the n. extensorius communicans passing through the radio-ulnar space. The muscles inserted on the forearm were associated

chiefly with movements of elevation and depression of the forearm at the elbow joint.

3. The less specialized chelonians have preserved the primitive gait, musculature and innervations, but the ectepicondylar foramen, primitively an open groove, has closed.

4. The urodeles have lost the tractor radii, and the ulnaris has moved to the ulnare. In *Necturus* the short extensors have been lost, in cryptobranchs the humerodorsalis has reached the phalanges and the dorsometacarpales have been lost.

5. The early amphibians, such as *Eryops*, still retained the tractor radii.

6. In anurans the ulnaris is split, the humerodorsalis reaches the phalanges, the radial group is highly specialized, the supinator manus reaches the humerus, and the motor nerves are reduced.

7. In *Sphenodon* the humerodorsalis insertions are reduced and the ulnaris is split into antibrachial and carpal parts, and reaches metacarpal V.

8. In lacertilians the humerodorsalis insertions are further reduced, and the extensor antibrachii ulnaris lost. The n. extensorius communicans supplies most of the muscles.

9. In crocodiles the extensor ulnaris is confined to the ulna, the humerodorsalis insertions reduced, the retractor lost, and the hand muscles specialized. All are supplied by the n. extensorius cranialis.

10. In mammals elevation and depression at the elbow joint have been lost. The tractor is missing, and the other radial muscles are specialized. The humerodorsalis extends to the phalanges and the ulnaris to metacarpal V, with an antibrachial part forming the anconeus. The n. extensorius cranialis becomes the motor part of the radial and the caudalis the n. to anconeus.

11. Though the nerve supplies to all muscles except those of the radial group are variable, the courses of the nerve trunks are constant except in anurans.

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KEY TO LETTERING

abd. pol. long.	abductor pollicis longus	*e. dig. com.*	extensor digitorum communis
anc.	anconeus	*e. dig. prof.*	extensor digitorum profundus
br. rad.	brachioradialis	*e. dig. uln.*	extensor digitorum ulnaris
carp. met.	carpometacarpalis	*e. rad. int.*	extensor radialis intermedius
Cen.	centrale	*e. rad. prof.*	extensor radialis profundus
dors. met.	dorsometacarpalis	*e. rad. sup.*	extensor radialis superficialis
e. ant. uln.	extensor antibrachii ulnaris	*e. uln.*	extensor ulnaris
e. carp. rad.	extensor carpi radialis	*e. uln. acces.*	extensor ulnaris accessorius
e. carp. rad. brev.	extensor carpi radialis brevis	*For.*	foramen
e. carp. rad. long.	extensor carpi radialis longus	*for. ectep.*	ectepicondylar foramen
e. carp. uln.	extensor carpi ulnaris	*hum. dors.*	humero-dorsalis
e. dig. brev.	extensores digitorum breves	*Int.*	intermedium
e. dig. brev. acces.	extensor s digitorum breves accessorii		
e. dig. brev. med.	extensores digitorum breves medii		

Int.-uln.	intermedio-ulnare	*n. to tr. rad.*	n. to tractor radii
lig.	ligament	*Pis.*	pisiform
n. dors. man. int.	n. dorsalis manus intermedius	*Rad.*	radiale
n. dors. man. rad.	n. dorsalis manus radialis	*Sca.-lun.*	scapho-lunar
n. dors. man. uln.	n. dorsalis manus ulnaris	*sup.*	supinator
n. ext. caud.	n. extensorius caudalis	*Sup. cr.*	supinator crest
n. ext. com.	n. extensorius communicans	*sup. man.*	supinator manus
n. ext. cran.	n. extensorius cranialis	*sup. man. acces.*	supinator manus accessorius
n. flex.	n. flexorius	*Tri.*	triquetrum
n. int. dors.	n. interosseus dorsalis	*tr. rad.*	tractor radii
n. int. vent.	in. interosseus ventralis	*Uln.*	ulnare
n. to anc.	n. to anconeus	*uln. met.*	ulno-metacarpalis

A RESURVEY OF THE ANATOMICAL FEATURES OF THE PILTDOWN SKULL WITH SOME OBSERVATIONS ON THE RECENTLY DISCOVERED SWANSCOMBE SKULL. PART II

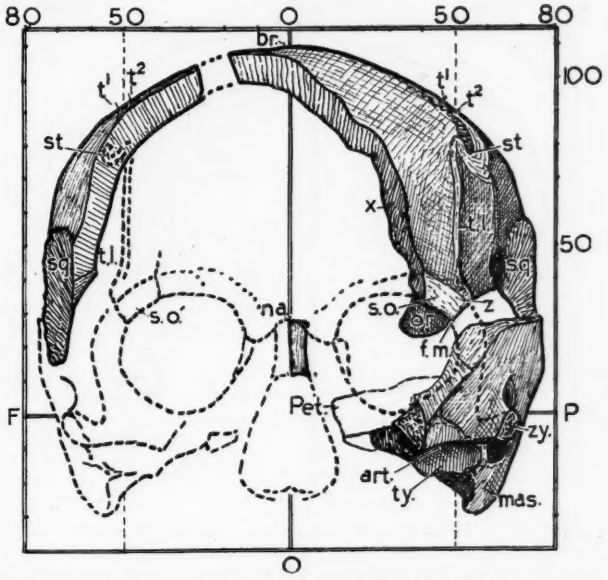
By SIR ARTHUR KEITH

A FRONTAL view of my Piltdown reconstruction is given in Text-fig. 19, made at right angles to the profile shown in Text-fig. 2. It will be observed that the temporal lines on the frontal are made to ascend almost vertically just outside the 50 mm. latitude, the minimum frontal width being 103 mm. This is the width, after many trials, which I was obliged to assume in 1913; after many attempts during the last six months I have reached the same result. In this I am in agreement with the reconstruction published by Elliot Smith in 1927 and with Friederichs's in 1932. Weinert (1933) places the frontal so that the temporal lines ascend inward as well as upward, giving a minimal width of 116 mm., agreeing with Smith Woodward's second reconstruction. In my reconstructions I contrived to have the apex of the petrous bone always in view (Text-fig. 19, *P*), keeping it within 14 mm. of the mid-sagittal plane of my reconstruction. Two other points I also kept constantly in my eye—namely the points marked t^1 and t^2 in Text-fig. 19, these points representing the upper and lower temporal lines immediately behind the coronal suture. The squamous margins of the parietals (*sq.sq.*) had also to tally. And as the width of the hinder end of the skull is fixed for all reconstructors at approximately 150 mm., the petrous can be brought into its appropriate relation with the mid-line only by altering the frontal width. In any attempt to make the forehead narrower than in Text-fig. 19, the bregmatic part of the left fragment becomes tilted upwards and assumes an impossible relationship to the right parietal. In width, the frontal bone of the Piltdown skull was as in modern man—save that it is relatively narrow as compared with the maximum width of the skull.

In the modelling of the fronto-malar process and in the conformation of its supraorbital ridge Piltdown man differed from all known kinds of mankind living and extinct. The supraorbital ridge is represented by a slightly raised band, 8 mm. wide and marked by striae running parallel to the orbital border, which is very sharp. Whereas the supraorbital region in all other forms of early man known to us is modelled as in the chimpanzee, in Piltdown man it follows the type seen during the childhood of the orang. The second frontal fragment, found by Mr Dawson in 1915, gives us the mesial termination of the supraorbital ridge and the isolated peculiar supraciliary ridge which, like the supraorbital, is but slightly raised from the surrounding bone, but its border is sharply delimited. From the frontal markings alone, I should infer that

Eoanthropus represents an early form of man belonging to a phylum altogether different from that represented by *Sinanthropus* and *Pithecanthropus*.

On the left fragment, part of a frontal boss is preserved (Text-fig. 19, above *x*). When the vault of the left fronto-parietal is viewed from above and behind, it is seen to be part of a frontal which had a high median ridge or keel, the elevation (as can be seen in profile, Text-fig. 2) being continued at the same level for some distance in front of the bregma. The forehead was upright and relatively high in the middle line, and equal to that of modern man in its width. It is in the frontal of the orang that we have to seek its prototype.

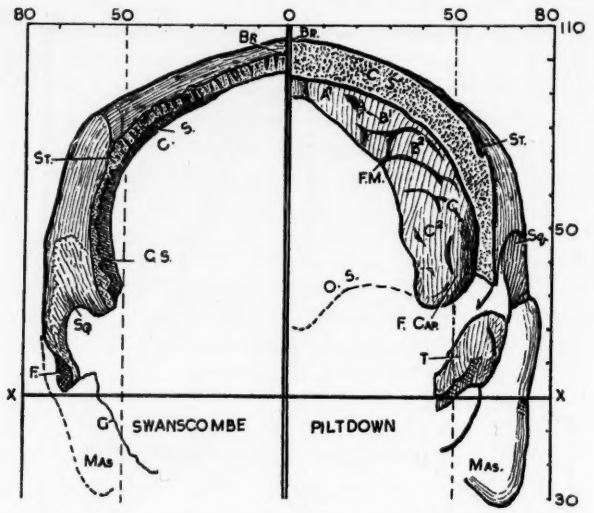


Text-fig. 19. Frontal view of the Piltdown skull made at right angles to the profile shown in Text-fig. 2. F.P., Frankfort plane. *OO*, mid-sagittal plane. The suggested outlines of the orbits are stippled. *br*, bregma; *st*, stephanion; *or*, fragment of orbital wall; *fm*, inner fronto-malar point; *so*, supraorbital ridge; *z*, fronto-malar process; *zy*, zygoma; *mas*, mastoid process; *pet*, apex of petrous (apicon); *art*, articular surface of temporo-mandibular joint; *ty*, tympanic plate; *tl*, anterior temporal lines; t^1, t^2, upper and lower temporal lines behind stephanion; *sq*, squamous articular surface of parietal; *x*, fractured edge of frontal.

Of the Swanscombe frontal we know only the margin which it articulated with the parietal. That margin is shown, composing the left half of the composite skull drawn in Text-fig. 20. In the left half is given a section of the left Piltdown parietal, made at its coronal suture. The greater thickness and mass of the Piltdown bone is at once apparent. But in their curvature, span and height the differences are not great. The intracranial width of the Swanscombe arch—as shown in Fig. 20—is 54 mm., against 57 mm. in Piltdown; but in height of intracranial arch Swanscombe is the greater.

In Text-fig. 20 is given the frontal aspect of the endocranial cast of Piltdown. The peculiar form of the part of the third frontal gyrus, known as the 'cap' (F. Cap.) is shown. *A* is placed on the upper frontal sulcus; A^1 on the upper frontal gyrus; *B*, B^1 are placed on the second or middle frontal, with the arcuate sulcus between them. *C*, C^2 are placed on the third frontal gyrus. *FM* I regard as the hinder end of the fronto-marginal sulcus, effecting a union with both arcuate and inferior frontal sulci.

None of those who have reconstructed the Piltdown skull have given a drawing of its right profile. It is easy to set up a theoretical left half; the



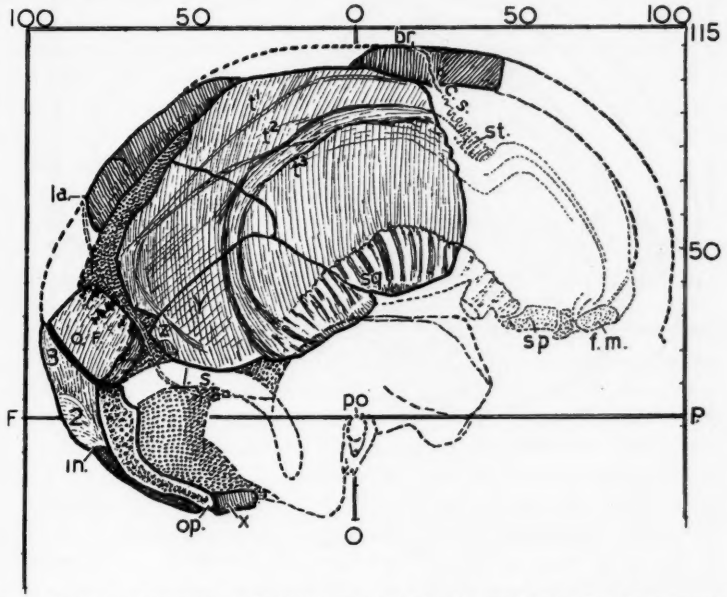
Text-fig. 20. On the right is shown a coronal section of the Piltdown skull, with the frontal area of the endocranial cast exposed. On the left, a frontal view of the Swanscombe skull (reversed). The Piltdown skull is orientated on the Frankfort plane (*X*–*X*); the drawing of the Swanscombe skull was made in my first series, when the skull was arranged so that the bregma was 4 mm. below the highest point in the vault. *cs*, coronal suture; *st*, stephanion; *br*, bregma; *sq*, squamosal suture; *F*, dovetailed process; *G*, mastoid margin of occipital bone; *os*, orbital surface. Further explanation is given in text.

difficulty begins when we attempt to place the right parietal fragment so that it will match in position the corresponding parts on the left.

In Text-fig. 21, I have reproduced a reversed outline of the left temporal bone; we have to poise the right parietal fragment so that its hinder lower border will articulate with the transposed temporal. The position given to this fragment has already been depicted in its occipital aspect (Text-fig. 9) and in its frontal aspect (Text-fig. 19). The position of the opisthion of the occipital (*op*), the fragment of the left lateral-wall of the foramen magnum (*X*), and the position of the lateral sinus (*ls*), are also indicated. So are the parts of the frontal with which temporal, sphenoid (*sp*), and malar (*fm*) articulate. The area

(*Y*) is flatter than on the left parietal. It will be observed that the opisthion is 42 mm. behind the mid-auricular plane (*OO*) and is 25 mm. below the level of the Frankfort plane (F.P.). The position of the coronal suture (*cs*) and stephanion is also shown, being copied from the left side.

In Text-fig. 22 there are shown, within the left profile of my Piltdown reconstruction, four series of structures. These are: (1) the endrocranial surface which is exposed by removal of the right parietal fragment; (2) a median section of the occipital bone with the parts of the endocranium in contact; (3) the

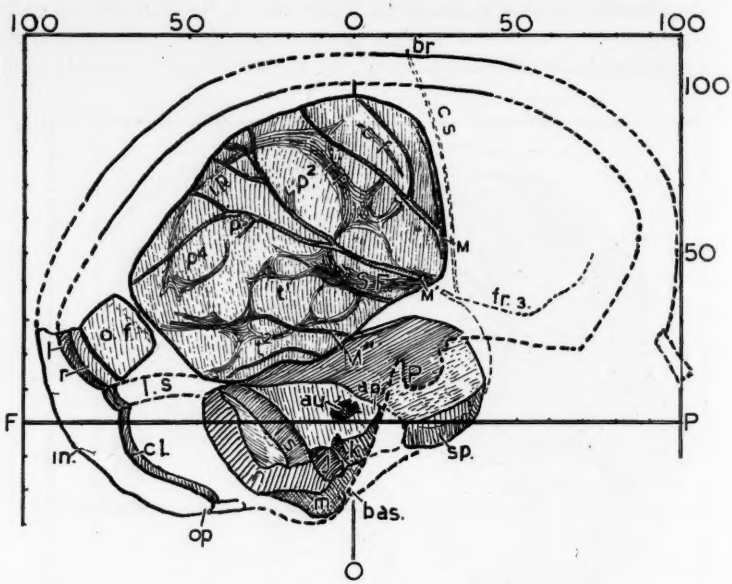


Text-fig. 21. The right aspect of the Piltdown skull with the outlines of the left temporal and frontal bones depicted in broken lines—traced from Text-fig. 2. *br, la, in, op, po,* as in Text-fig. 2; t^1, t^2, t^3, the lower, upper and supreme temporal lines; *sq,* squamous margin of parietal; *st,* stephanion; *cs,* coronal suture; *ls,* lateral sinus; *of,* occipital fragment; *fm,* fronto-malar sutures; *sp,* articulation for sphenoid; 2, 3, the lower and middle occipital ridges, indicated in Text-fig. 9; *X,* lateral wall of foramen magnum; *Y,* supra-asterionic field; *Z,* perforation made by workman's pick.

inner aspect of the left temporal with its sutural contacts demarcated; (4) a suggested reconstruction of the base.

Taking the endocranial markings first, one notes many points in which the convolutionary pattern differs from that of the left side; the manner in which the meningeal vessels divide is different. The sylvian fissure (*SF*) is wide, shallow and short; the central (*cf*) and intraparietal fissures—both ascending and horizontal limbs (*ip*) are identified; p^2, p^3, p^4, I suppose to represent the three usual divisions of the inferior parietal lobule. The impression of the

first (t^1) and second (t^2) temporal gyri are extensive. Behind the temporal gyri comes a flat parieto-occipital field; the part of the occipital lobe under the right fragment (*of*) is also flat and in conformity with the parieto-occipital field. The left occipital pole (*l*) is seen to project 4 mm. behind the right (*r*). On the other hand, the right cerebellar lobe (*cl*) projects behind the left.



Text-fig. 22. Right profile of the Piltdown skull with the endocranial cast from the right parietal and the mesial aspect of the left temporal in position. An outline of a section of the base of the skull is also suggested by an interrupted line. F.P. Frankfort plane; *OO*, mid-auricular vertical plane.

Endocranium: *SF*, sylvian fissure; *cf*, central fissure; *ip*, *ip*, intraparietal fissure, ascending and horizontal limbs; p^2, p^3, p^4, identified as supramarginal, angular and post-parietal gyri of inferior parietal; t^1, t^2, first and second temporal gyri; *fr* 3, position of third frontal convolutions; *M*, post-coronal branch of middle meningeal; *M'*, branch of middle meningeal; *M''*, on inner aspect of left squamosal with pointer to the meningeal artery.

Mesial aspect of left temporal bone: *au*, internal auditory meatus; *ap*, apicon; *ls*, lateral sinus; *m*, mastoid process; *k*, area on petrous which made contact with basi-occipital; *j*, for jugular sinus; *n*, sutural area for contact with the occipital; *sp*, sutural surface for sphenoid; *P*, suggested position of pituitary fossa; *bas*, basion.

Occipital: *of*, occipital fragment; *l*, left occipital pole; *r*, right pole; *cl*, right cerebellum; *in*, inion; *op*, opisthion.

Coming to the temporal bone we note that the internal auditory meatus (*au*) lies just above the F.P. Lt.-Col. Lack (1931) found that the mean position for skulls of all human races and types was a little lower—the F.P. cuts the meatus so that the lower margin of the meatus lies nearly $\frac{1}{2}$ mm. below the F.P. On the other hand, he found that the lateral sinus at the asterion rises only

5 mm. above F.P. in dolichocephalic skulls; 10 mm. in brachycephalic skulls. In Text-fig. 22 this point of the sinus is 12 mm. above the F.P. In these markings the Piltdown reconstruction conforms to modern proportions.

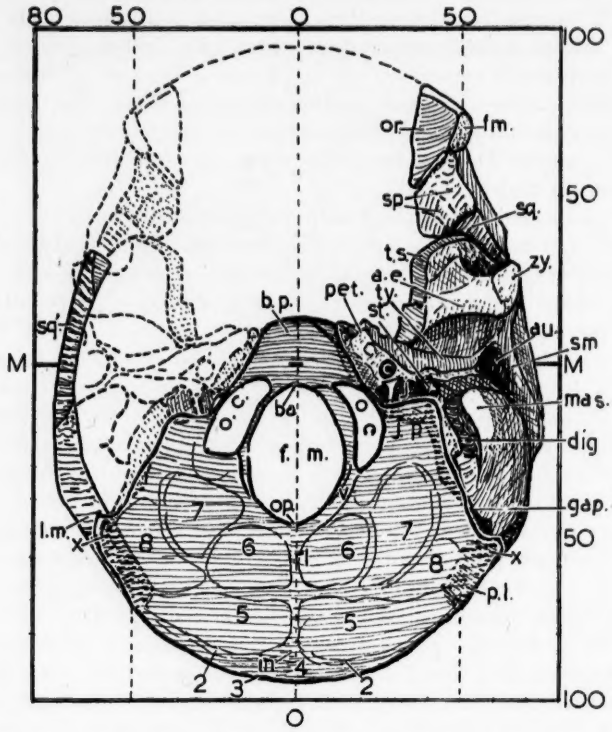
I attach importance to the position of the point on the apex of the petrous bone (*ap*) which I have named the apicon. In Text-fig. 22 the apicon is seen to be placed 5 mm. above F.P. and 9 mm. in front of the mid-auricular vertical plane—a very common position in modern skulls. The apicon gives the upper margin of the basilar process at, or near, the junction of basi occipital with basi sphenoid. The position of the apicon shown in Text-fig. 2 is a very common one in modern skulls.

Important as guides in reconstruction are the sutural surfaces seen on the mesial aspect of the temporal bone. They are indicated in Text-fig. 22; *sp* is for the great wing of the spheroid; *k*, for the basilar process; *j* is the jugular fossa; *n* is for the occipital bone—from jugular process up to asterion. The inner aspect of the mastoid process (*m*) is also seen.

The basion is shown—22 mm. below the porion and giving the foramen magnum a length (*ap*, diameter) of 42 mm.—slightly greater than in the Swanscombe skull (40 mm.), in which the foramen is intact. The position of the pituitary fossa is suggested; its floor is seen to be 10 mm. above F.P. The position of the fossa, bounded laterally by the squama of the temporal, and its relationship to the floor of the middle fossa, conform to the relationship found in modern skulls. It will be seen that the opisthion is 3 mm. below the level of the inferred position of the basion and that the foramen magnum looks downwards and slightly forwards. This aspect I shall return to later (p. 243). The length of the base of the skull (*bas.-nas.*) in the reconstruction shown in Text-fig. 22 is 107 mm.—a measurement often surpassed in modern skulls of a primitive type. The length of the basilar process and the angle which its intracranial surface forms with the Frankfort plane are dealt with on p. 244.

To prove the probability that any reconstruction is approximately right, it is necessary to test the basal aspect of the reconstruction. In Text-fig. 23 I have fitted the basal aspect of the Swanscombe occipital into one of the several trial reconstructions I have made from the Piltdown skull. In both cases, the views presented have been drawn at right angles to the F.P. In the particular reconstruction of Piltdown shown in Text-fig. 23, I attempted to give a minimal frontal width of 98 mm.; a maximal parietal width of 148 mm., bringing the apex of the petrous within 13 mm. of the mid-line of the base. I found that the Swanscombe occipital fitted into this reconstruction surprisingly well. The gap (Text-fig. 23) between the mastoid and occipital became very narrow; all the parts of the Swanscombe occipital made appropriate contacts with the mastoid and petrous parts of the Piltdown temporal. The jugular process (*jp*) comes just behind the notch for the jugular vein (*j*). I had to abandon this particular reconstruction of the Piltdown skull because of discrepancies which appeared along the vault. Still, the result convinced me that in giving the

occipital of Piltdown the same relationship to the parietal as was found in Swanscombe, I was moving along a correct course.

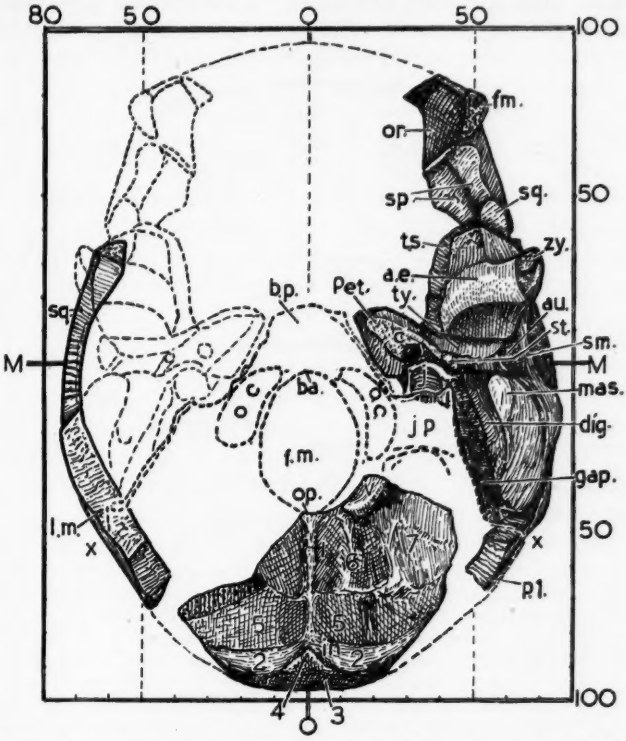


Text-fig. 23. The basal surface of the Swanscombe occipital, drawn at right angles to the F.P. It is fitted into a special (narrow) reconstruction of the Piltdown fragments—also drawn at right angles to the F.P. *OO*, median sagittal plane of base; *MM*, mid-auricular plane. Explanation proceeding from the frontal to the occipital end: *or*, orbital surface of frontal; *fm*, fronto-malar process; *sp*, articular surface for great wing; *sq*, squamosal surface on frontal; *zy*, zygoma; *ts*, temporo-sphenoid suture; *ae*, articular eminence; *ty*, anterior surface of tympanic plate; *st*, root of styloid; *pet*, apex of petrous; *au*, external auditory meatus; *sm*, suprameatal ridge; *mas*, apex of mastoid; *dig*, digastric fossa; *gap*, interval between mastoid and occipital borders; *c*, carotid foramen; *j*, opening for jugular vein; *bp*, basilar process; *oc*, occipital condyle, *jp*, jugular process; *fm*, foramen magnum; *ba*, basion; *op*, opisthion; *sq'*, squamous border of right parietal; *lm*, lamboid margin of the same; *pl*, lambdoid margin of left parietal; *in*, inion; 1, external occipital crest; 2, lower curved ridge; 3, middle curved ridge; 4, supranuchal area; 5, area of insertion of complexus; 6, 7, areas for recti muscles; 8, area for superior oblique.

A basal view of the reconstruction of Piltdown I finally adopted (Text-fig. 2) is shown in Text-fig. 24. The stippled outline of the foramen magnum and of the occipital condyles depicted there are those of Swanscombe; in that occipital

the foramen magnum is of large dimensions—namely 40 mm. long by 30 mm. wide. In Piltdown, I presume the dimensions to have been still greater—42 mm. long and 32 mm. wide—for it will be seen that the ridged lateral margin (*V*) lies beyond the Swanscombe outline.

We have seen that the maximum width of the occipital of Swanscombe is 124 mm.; in Piltdown 132 mm. Measured between the most lateral points of

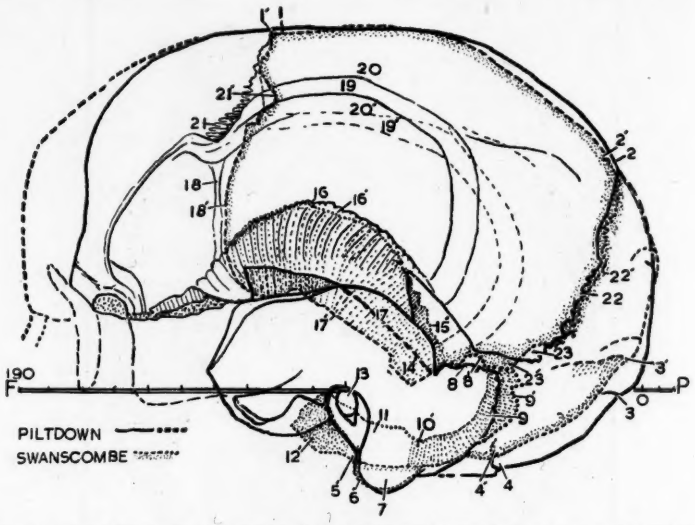


Text-fig. 24. Basal view of the final reconstruction of the Piltdown skull—at right angles to the plane shown in Text-fig. 2. The stippled outline of the foramen magnum and adjacent parts of the occipital are taken from the Swanscombe outline, shown in Text-fig. 23. The letters and numbers have the same significance as in Text-fig. 23.

its jugular process—the occipital of Swanscombe has a width of 84 mm.; the width of Piltdown in the same region is 90 mm. It will be seen in Text-fig. 24, where the mastoid margin of the jugular process of Swanscombe is represented by a broken line, that a gap is left between this margin and the adjacent mastoid of Piltdown. In Piltdown the width in the mastoidal region was very great; the apices of the mastoid process were 120 mm. apart; the bases, at the insertion of the sternomastoid, were 142 mm. apart—at least 6 mm. more than in Swanscombe.

On the other hand the area for attachment of the muscles of the neck, from opisthion to inion, was greater in Swanscombe. A comparison of Text-figs. 23 and 24 proves that the areas for muscular insertion are similar in Piltdown and Swanscombe, but the complexus in Swanscombe must have been more strongly developed than in Piltdown. The unexpected short distance from opisthion to inion in Piltdown is a matter I shall return to presently.

The basal view brings out certain remarkable features of Piltdown man. There is first the massive form of its short fronto-malar process (*fm*); the



Text-fig. 25. Superimposition of a profile of the Swanscombe parietal and occipital (broken line) upon the corresponding parts of the Piltdown profile. F.P., Frankfort plane. The skulls are superimposed so that the upper arches of the parietals correspond. 1, 1′, bregma of Piltdown and of Swanscombe; 2, 2′, lambda; 3, 3′, inion; 4, 4′, opisthion; 5′, basion; 6′, occipital condyle; 7, mastoid; 8, 8′, parieto-mastoid suture; 9, 9′, masto-occipital suture; 10′ occipital showing articulation for mastoid; 11′, for jugular process of occipital; 12′, basi-occipital (stippled); 13, ext. auditory meatus; 14′, on dove-tailed process of squamous margin; 15′, 16, 16′, upper margin of squamous articulation; 17, 17′, lower border; 18, 18′, coronal border; 19, 19′, 20, 20′, lower and upper temporal lines; 21, 21′, stephanion; 22, 22′, lambdoid suture; 23, 23′, asterion.

wide rough area on the frontal for the articulation of great wing (*sp*); the massiveness and strength of the articular eminence (*ae*) and a tympanic plate (*ty*) modelled as in modern man; a particularly small digastric fossa (*dig*) and a relatively small foramen for the internal carotid artery (*c*). Transverse measurements omitted from my table may be estimated from Text-fig. 24 (reproduced, as are all the drawings, half natural size).

I now return to answer a question mentioned at the beginning of this article: How far does the relationship between occipital and parietal in the Swanscombe skull help us in placing the fragment of the Piltdown occipital in its proper

relationship to the parietal? The answer is given in Text-fig. 25 where the Swanscombe bones (broken lines) are placed on the reconstructed Piltdown bones. The upper arches of the parietal are superimposed so that the bregma of Piltdown (1) is adjacent to that of Swanscombe (1′); the lambda of the one is adjacent to that of the other (2, 2′). The coronal (18, 18′) and lambdoid (22, 22′) borders agree. The borders which do not agree are the lower or squamous in their hinder parts (17, 17′), that of Swanscombe being 5 mm. nearer the meatus than that of Piltdown. Hence the temporal bone of Piltdown cannot be fitted into the space between the parietal and occipital of Swanscombe.

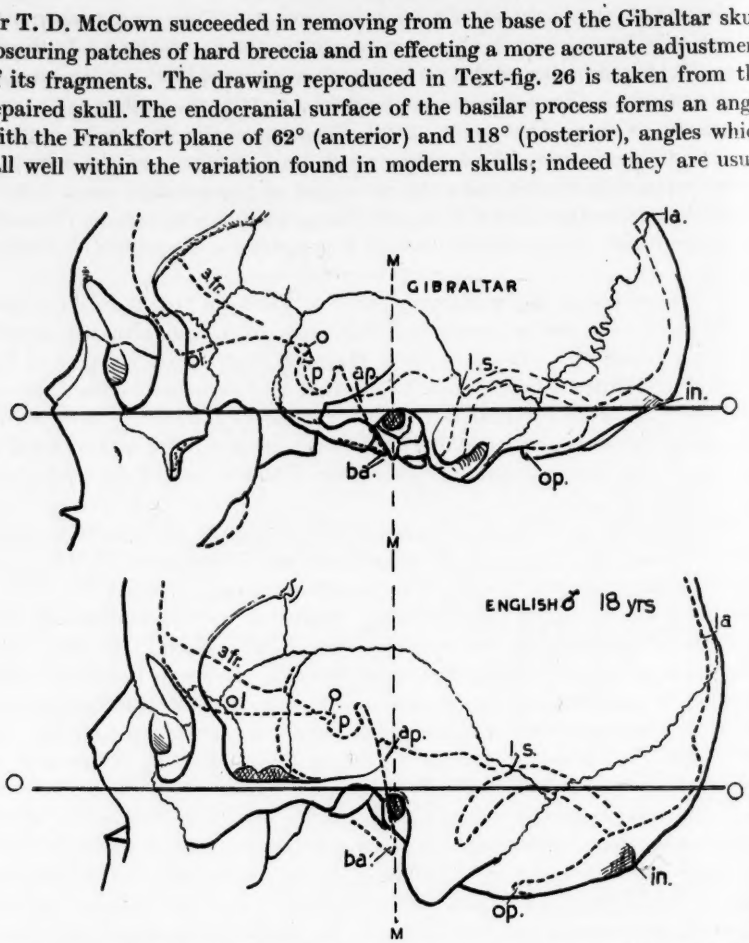
As superimposed, the auditory meatus of Piltdown (13) is in its proper relationship to the basilar process of Swanscombe (12′), being situated 21 mm. above the basion (5′). It will be seen, however, that the nuchal part of the occipital of Piltdown falls 5 mm. below that of Swanscombe; the inion of Piltdown falls below the Frankfort plane, that of Swanscombe 7 mm. above this plane. When the occipital of Piltdown is pushed upward and forward to the position occupied by that of Swanscombe, a deformation of the cerebellum is produced.

The explanation of the discrepancy in the position of the occipitals seems to be as follows. Suppose we depress the hinder part of the parietal of Piltdown until its lower border (17) comes to lie over that of Swanscombe (17′). At the same time the temporal bone with the meatus is carried downwards and forwards; so too would be the basilar process of the occipital; the rest of the occipital bone would follow in the same direction. In short, if we are to get guidance from Swanscombe as to the proper position of the occipital of Piltdown, then we have to superimpose not the upper borders of the parietals but their lower. When we do this the relation of the occipital to the parietal of Piltdown becomes that shown in Text-fig. 25.

To find an occiput of this low kind was the last thing I expected in the skull of early man. All through my reconstructions of 1913, I was confident it must have the form found in Neanderthal skulls—particularly in the Gibraltar skull, in which the occiput is modelled on simian lines. To explain the conclusion I have been obliged to draw, let me say I find it necessary to distinguish between two opposite occipital conformations which are found in human skulls —with every grade between the two extremes. The contrasted types are illustrated in Text-fig. 26, the upper that of the Gibraltar skull, the lower that of an English youth, both skulls being in the Museum of the Royal College of Surgeons in England. In the Gibraltar skull the nuchal surface of the occiput is flat, the opisthion is at a slightly higher level than the basion; the inion is high. In short, the occiput is turned upwards—is *anatreptic*. In the lower drawing, all is the opposite; the condition may be called *catatreptic*.

In the anatreptic skull the upper surface of the basilar process is less upright than in the catatreptic type. Prof. G. L. Sera (1909) drew attention to the inclination of the basilar process of the Gibraltar skull. In 1937

Mr T. D. McCown succeeded in removing from the base of the Gibraltar skull obscuring patches of hard breccia and in effecting a more accurate adjustment of its fragments. The drawing reproduced in Text-fig. 26 is taken from the repaired skull. The endocranial surface of the basilar process forms an angle with the Frankfort plane of 62° (anterior) and 118° (posterior), angles which fall well within the variation found in modern skulls; indeed they are usual



Text-fig. 26. Profile of the Gibraltar skull with a section of the base represented by a broken line. Below it is a corresponding drawing of a modern skull—that of an English youth aged 18 years. *OO*, Frankfort plane. The lettering from before backward: 3 *fr*, lower border of third frontal gyrus; *ol*, cribriform plate; *o*, optic foramen; *p*, pituitary fossa; *ap*, apicon of petrous; *ba*, basion; *ls*, lateral sinus; *op*, opisthion; *in*, inion; *la*, lambda.

angles. The angle formed by the plane of the foramen magnum, which is almost parallel with the F.P., is 121°. In the English skull (Text-fig. 26) the basilar angle is 98° (posterior), the basilar process being nearly upright, while the angle with the plane of the foramen magnum is 117°. The basilar process, being preserved in the Swanscombe skull, we can determine the angle of its endocranial surface. Its inner surface, which is 21 mm. in length (basi-

occipital), forms an angle (posterior) of 118° with the Frankfort plane—being thus the same as in the Gibraltar skull. The angle formed with the plane of the foramen magnum is 125° (as determined on my drawings), being the same as Mr Marston's measurement. In the reconstruction of the Piltdown base (Text-fig. 22) the angle with F.P. measures 112°, that with the foramen magnum 118°. Thus the total angle formed by basilar process and foramen magnum remains fairly constant in all four skulls: Swanscombe 125°, Piltdown 118° (estimate), Gibraltar 121°, modern English 117°. On the other hand the angle formed with F.P. varies from 118° in Swanscombe, 118° Gibraltar, 112° Piltdown, 98° English. The more vertical form of the basilar process seen in catatreptic skulls is the least simian—the more neoanthropic. Piltdown falls in the catatreptic group. In this respect Swanscombe is the more simian or primitive.

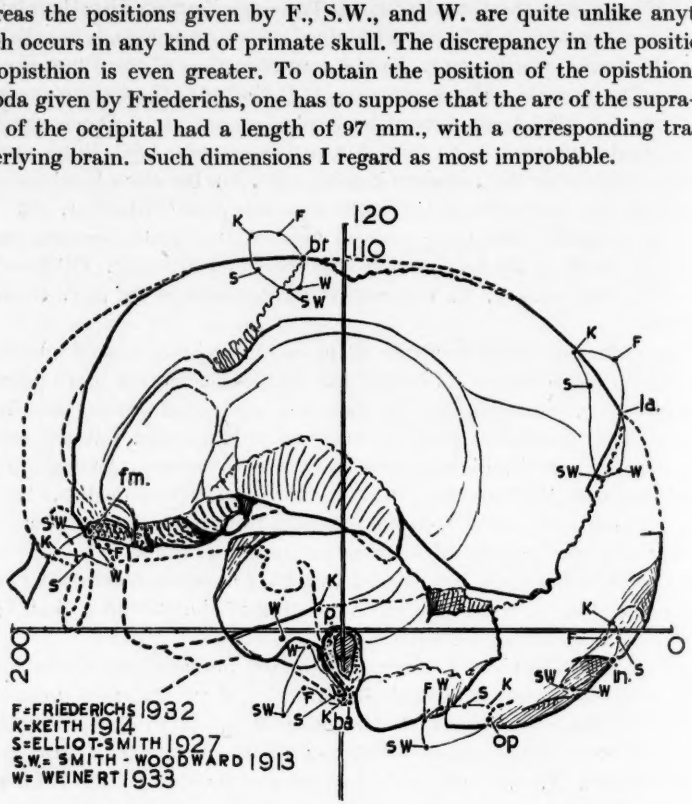
I regard the catatreptic condition of the occiput as being a foetal inheritance —one which has persisted into adult life, thus replacing the more primitive anatreptic type. Such features are spoken of as "pedomorphic" and in this feature as in several others Piltdown man was pedomorphic. Catatrepism and anatrepism are not reliable as guides to race or to species. Amongst the Mt Carmel people Mr McCown and I found both types with intermediates between them. Mr Dawson's second occipital fragment from Piltdown must have been like the Swanscombe occipital—part of an anatreptic skull.

In a series of drawings, Text-figs. 27, 28, 29, I have summarized the extent and the direction in which my reconstruction of the Piltdown skull (1938) differs from some former reconstructions

In Text-fig. 27 is given a drawing of the left profile of my reconstruction (1938), with two planes indicated—Frankfort and vertical mid-auricular. I have made similar drawings of the reconstructions of Friederichs (1932), of Keith (1915), of Elliot Smith (1927), of Smith Woodward (1913) and of Weinert (1933). The two planes just mentioned provide a basis from which comparative measurements can be made. For example, the bregma (*br*) in the present reconstruction is 110 mm. above F.P. and 12 mm. anterior to mid-auricular plane. The initials K., F. etc. in Text-fig. 27 give the position of the bregma in other reconstructions. It will be observed that, as regards height of vault—indicated by the position of the bregma, the reconstructions fall into three groups—the high, the medium and the low. K. and F., abiding by anatomical indications, obtained a high vertex (117, 118 mm.); S.W. and W., following other indications, obtained a height of 100 mm.; S. and K. (1938) are intermediate. The distance in front of mid-auricular plane depends on the manner in which the temporal is articulated with the parietal—particularly on the angle at which it is articulated.

The variation at the lambda (*la*) is of the same kind but greater in extent—the difference between highest (F.) and lowest (S.W.) being 36 mm. So too with the position of the inion (*in*). The most important differences concern the basion (*ba*) and opisthion (*op*), the anterior and posterior limits of the foramen

magnum. As regards the basion, Elliot Smith and I are in close agreement, whereas the positions given by F., S.W., and W. are quite unlike anything which occurs in any kind of primate skull. The discrepancy in the position of the opisthion is even greater. To obtain the position of the opisthion and lambda given by Friederichs, one has to suppose that the arc of the supra-inial part of the occipital had a length of 97 mm., with a corresponding tract of underlying brain. Such dimensions I regard as most improbable.



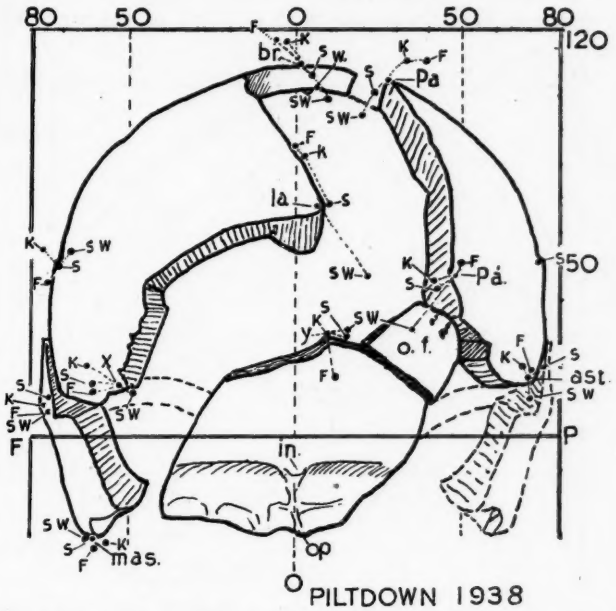
Text-fig. 27. A left profile of the Piltdown skull (Keith, 1938) showing how far it differs from former reconstructions. It is set on the Frankfort plane (0–200), with mid-auricular plane indicated by vertical line. Explanation in text.

The position given to the apex of the petrous bone (*P*) is shown in Text-fig. 27; Weinert has placed it first below the F.P. and 18 mm. in front of mid-auricular plane, an impossible position. The variations in the position of the mesial end of the fronto-malar (*fm*) are also considerable.

In Text-fig. 28 is summarized the reconstructional variations—seen in the coronal plane of an occipital view. As regards the position of the apex of the mastoid (*mas*), the lateral eminence (supramastoid) of the temporal, the lateral parietal point (at the upper margin of the squamous articulation), and of the asterion, there is a welcome measure of agreement. But as regards the position of bregma and lambda, there are the same wide differences as were seen in the profile view of these points (Text-fig. 27). The widely different positions given

to the upper angles of the right parietal (*Pa, Pa'*) are also depicted in Text-fig. 28.

In Text-fig. 29 are depicted the reconstructional differences in a coronal plane—this time in a frontal view. The differences given to the bregmatic projection of the left fronto-parietal (*br*) and to the corresponding angle of the right parietal (*an*) are again illustrated. The most noteworthy discrepancy relates to the position of the minimal frontal point (*fr*); F., S. and K. are in agreement, whereas W. and S.W. place it much more laterally, increasing the lower frontal width and decreasing the upper frontal width. Similarly, too,



Text-fig. 28. Occipital view of the Piltdown skull (Keith, 1938), the F.P. and mid-sagittal planes being indicated. Explanation in text.

with the position of the fronto-malar point (*fm*). As regards bizygomatic width (*zy*) there is not a great discrepancy. The variation given to the position of the anterior inferior angle of the right parietal (*pt*) is remarkably great. It will be noted that the position I give to it in my present reconstruction is also the same as that assigned to it by Elliot Smith.

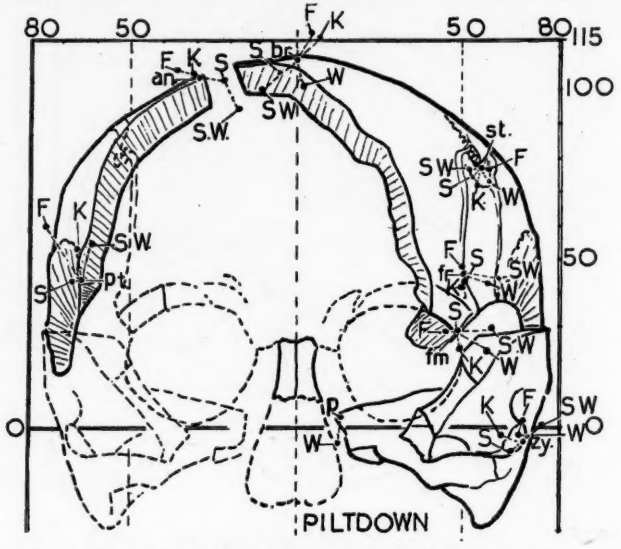
Thus it will be seen that the widest range of opinion lies (1) in the extent to which the right and left fragments should be approximated along the vault; the more they are approximated the lower the vault and the smaller the cranial capacity; (2) the position which should be given to the occipital bone.

My reconstruction of 1938 differs from the one made in 1914 (1) in lowering

the auricular height of the vault from 117 mm. to 110 mm., and (2) in giving the occipital bone another relationship to the parietal and temporal—a relationship which is based on that which it occupies in the Swanscombe skull.

To the account of the mandible I have nothing to add or withdraw to what I have already given (Keith, 1915), except to say that all the studies I have made of the cranium since 1915—particularly of its temporo-mandibular and sphenoid regions—have convinced me more firmly than ever that skull and mandible are in harmony, and formed parts of the same head.

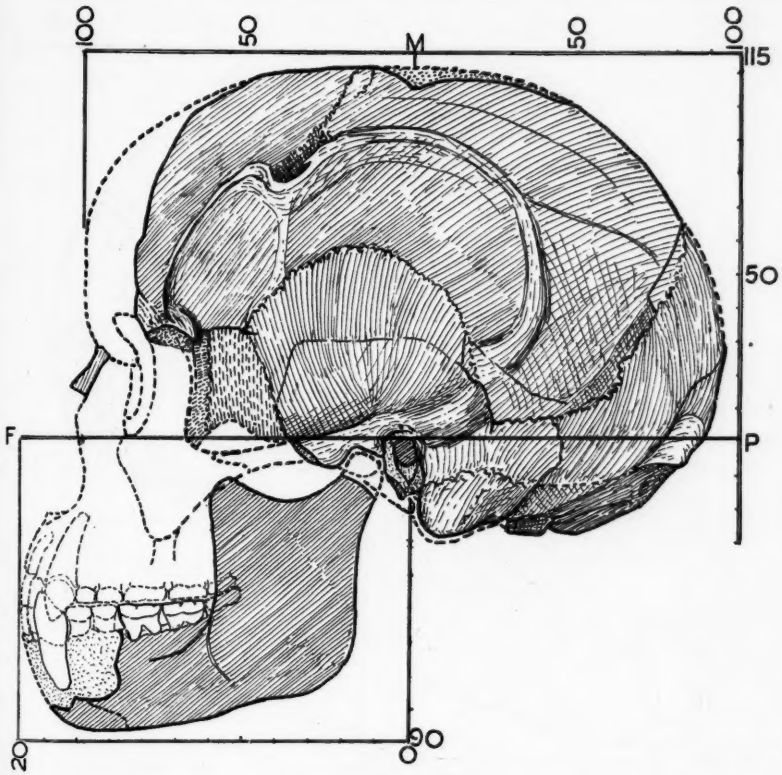
It may be well, however, to complete my account by giving a profile drawing of the skull as a whole. In Text-fig. 30, I have extended the reconstruction shown in Text-fig. 2—one with a length of 194 mm. and a vault



Text-fig. 29. Frontal view of the Piltdown skull, (Keith, 1938), with the same planes as in Text-fig. 28. Explanation in text.

which rises 110 mm. above the Frankfort plane. I have completed the squamous plate of the temporal, filled in the great wing of the sphenoid and the orbital plate of the malar. The gap between parietal and occipital is also filled up and the profile of the face outlined. Certainly the prognathism is marked, and although such a full muzzle has never been seen before in a skull that is human, yet the degree of prognathism is not beyond the range found in Australian and African skulls. Another reconstruction of an experimental nature is given in Text-fig. 31. In Text-fig. 8 I gave an occipital view of a reconstruction based on a strict observance of mid-line markings—but which, for reasons already given, I had to abandon. In Text-fig. 31 I give the left profile of that reconstruction with two additions: I have placed in the forehead a drawing of

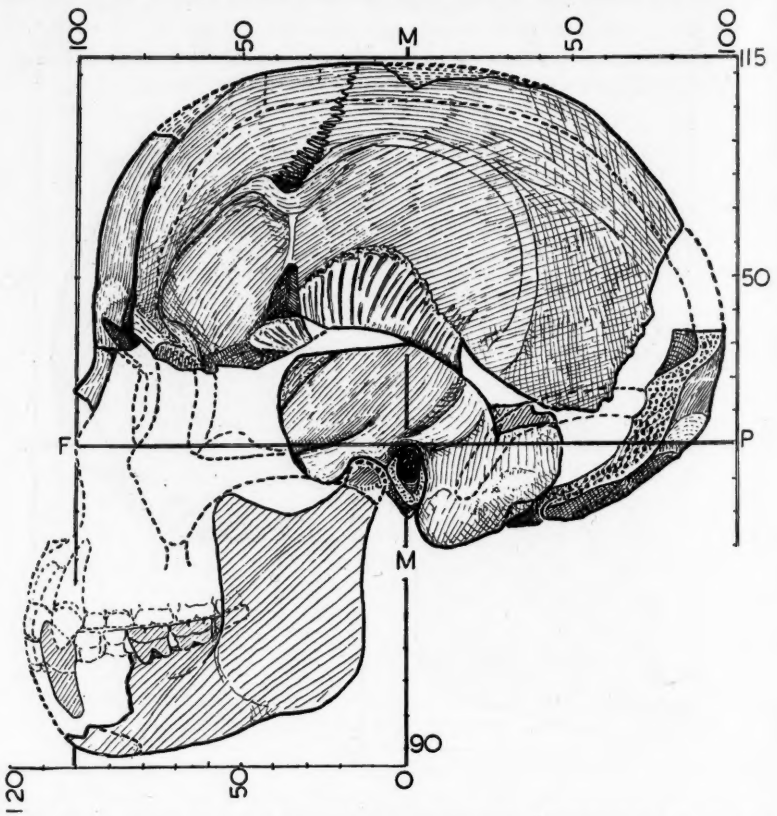
the frontal fragment found by Mr Dawson in 1915, seen in true profile. I have articulated with this fragment the nasal bones of No. 1. The outline of the forehead thus depicted runs directly into the bridge of the nose; there is no nasal or subglabellar notch. It is certainly an illegitimate procedure to fashion a reconstruction out of fragments belonging to separate individuals, yet it is one not without interest. It serves to bring home another of the peculiar



Text-fig. 30. Reconstruction of the completed Piltdown skull, seen in left profile—the reconstruction depicted in Text-fig. 2. The total length as drawn is 194 mm.; it is possible that a too liberal addition has been given to the missing frontal part (see Text-fig. 31).

features of Piltdown man. Another minor alteration in the reconstruction shown in Text-fig. 31 is a slight alteration in the position of the occipital fragment; it has been moved more towards the position occupied by the occipital bone in the Swanscombe skull than is shown in Text-fig. 30. In this reconstruction the maximum length is 191 mm.; its width 150 mm.; its auricular height 113 mm.

One important matter requires a brief consideration. What place are we to assign to Swanscombe man—so far as we know him from the hinder half of his skull and endocranial cast—in the phylum of evolving pleistocene humanity? Geologists are unanimous as to his position in time; he belongs to the latter end of the Mindel-Riss interglacial. He is therefore older in time than all the fossil remains of Neanderthal man that have been found in Europe—with one

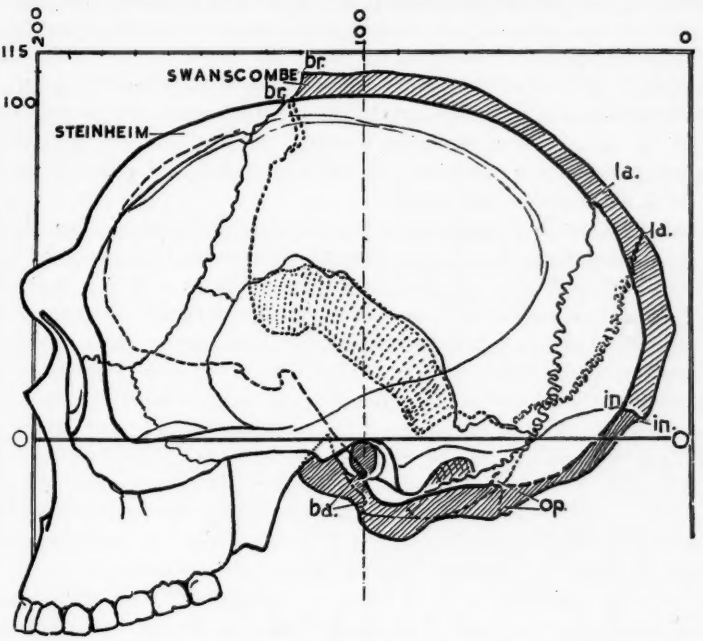


Text-fig. 31. Another reconstruction—based on that illustrated in Text-fig. 8. The forehead has been completed by introducing the frontal fragment of another individual, found by Mr Dawson in 1915. To this has been articulated the nasal bones of the original skull. The total length is 191 mm.; the height of the vault above F.P. 113 mm.

important exception—the Heidelberg mandible, which has all the indications of representing an early Neanderthal stock. This mandible must be attributed to the latter part of the Günz-Mindel interglacial. Piltdown man is attributed to—and in my opinion rightly attributed to—an early phase of the same interglacial.

Is Swanscombe man to be attributed to the Neanderthal phylum, or to the totally different Piltdown stock?—or does he represent a third phylum—one with a near relationship to modern man? Prof. Le Gros Clark and Dr Morant (1938) have expressed the opinion that in their main metrical characters the Swanscombe occipital and parietal bones "fall well within the range found in *H. sapiens*".

Seeing that in the earlier forms of Neanderthal man—such as that from Steinheim (Würtemberg), described by Berckhemer (1934) and by Weinert (1936) and from Mt Carmel by McCown and Keith (1938) the occipital end of



Text-fig. 32. Profile of the Steinheim skull, after Dr Weinert's reconstruction. On it is superimposed a shaded outline of the Swanscombe skull. Both are orientated on the F.P.

the skull is full and shaped as in modern man, it might be that Swanscombe man may prove to have a Neanderthal forehead. I am not of this opinion. In Text-fig. 32 I have superimposed my drawing of the Swanscombe bones on the corresponding parts of the Steinheim skull—the outline of the latter being taken from D. Hans Weinert's reconstruction. Both are orientated on the same plane (F.P.) and corresponding parts have been brought into juxtaposition. In the Steinheim skull, as in all skulls of the Neanderthal species, there is a wide spheno-parietal contact. In Swanscombe there is none. In Steinheim, the nuchal occiput is flat and shallow just as in the Gibraltar skull.

The Swanscombe skull differs altogether from the Steinheim skull; it is not of the Neanderthal type.

Now, if we did not know of Piltdown man we should be content to assign Swanscombe man to the modern species—*H. sapiens*; he would be, if we accepted this view, the earliest representative of *H. sapiens* known to us. A large part of this paper has been devoted to an exposition of the many anatomical resemblances in the Swanscombe bones to corresponding parts of Piltdown. Taking into consideration the evidence as it now stands it seems to me that the most probable explanation is to regard Swanscombe man as a member of the Piltdown phylum—probably greatly changed from the older type, for the time interval between Piltdown and Swanscombe is an immense one. Also I regard the "London skull" described by the late Sir Grafton Elliot Smith (1927) and by myself (1931) as a member of the Piltdown group. The Bury St Edmunds cranial fragment (Keith, 1913) is probably of the same period and of the same race as Swanscombe man. Dr Matthew Young (1938) has indicated the points in which the London cranium resembles the Swanscombe and Bury St Edmunds specimens.

Such an inference presumes that in early pleistocene times, when the well-known Pithecanthropic and Sinanthropic types were being evolved in the East, a totally different type, that of Piltdown, was in existence in the West and that this type continued to survive and change until mid-pleistocene times, Swanscombe, London and Bury St Edmunds being descendants of the type. We have knowledge of the type only in England as yet. This type was a mosaic in which neanthropic and some simian features were strangely blended. Such a supposition is the most probable explanation of the facts now at our disposal.

I have said nothing of the probable sex and age of the two individuals whose fossil fragments are dealt with in this paper. The Swanscombe individual I presume to have been a male under 25 years of age. The Piltdown individual is older, over thirty years of age, but as to sex I am still not quite certain, but on the whole its characters seem to me to be those of a male.

One other character I have failed to emphasize. Both Piltdown and Swanscombe may be said to be thick-walled skulls but the thickness of Piltdown is the greater and it is uniform; there is no modelling; it is made to withstand pressures passing in all directions. The bones of Swanscombe are modelled—are more evolved. The mean thickness of Piltdown is 3 mm. greater than in Swanscombe.

SUMMARY

The anatomical characters of the Piltdown and Swanscombe skulls are considered and reconstructions of their original forms attempted.

The Swanscombe skull is estimated to have had a length of 185 mm., a width of 144 mm., an auricular height of 110 mm. and a cranial capacity of 1350 c.c.

An analysis of its anatomical features has led me to infer that Swanscombe

man is probably a mid-pleistocene representative or descendant of the early pleistocene Piltdown type.

The anatomical characters of the Piltdown skull have been re-examined and several of a simian nature, which appeared to have escaped notice hitherto, are described.

It is concluded that the Swanscombe skull provides a clue to the position which must be given to the occipital fragment in a reconstruction of the Piltdown skull.

A re-examination of the endocranial fragments gives unmistakable evidence of asymmetry in the left and right hemispheres of the brain—to a degree beyond anything known in the brain of modern man.

This asymmetry also affected the skull so that the sagittal suture and the internal median crest of the occipital lay to the right of the mid-sagittal plane of the head. In reconstruction allowances have to be made on account of the asymmetry.

It is only when this asymmetry is recognized that a harmonious reconstruction is possible.

The reconstruction illustrated in this paper gives the Piltdown skull a length of 194 mm., a parietal width of 150 mm., an auricular height of 110 mm. The endocranial cast taken from this reconstruction displaced 1358 c.c. of water.

Certain features of the forehead and occiput of Piltdown man are interpreted as pedomorphic in nature.

ACKNOWLEDGEMENTS

These investigations which have occupied the author for the greater part of the past twelve months would not have been possible without the co-operation of Prof. John Beattie, Conservator of the Museum of the Royal College of Surgeons, England. To him and to the Assistant Conservator, Dr A. J. E. Cave, I am indebted for placing freely at my disposal the rich supply of anthropological material in their charge. I am also indebted to Mr F. Watson, laboratory assistant at the Buckston Browne Farm, where my investigations were carried out, for preparation of photographs and lantern slides.

TABLE OF MEASUREMENTS BY VARIOUS AUTHORS

	Martin No.	S.W.	E.S.	F.	W.	K.	Swan.
Length, max.	1	196·5	186	189	186	194	185
Width, max.	8	155	152	152	156	154	(146)
,, bi-parietal	—	149	150	148	—	150	144
Height, ba.-br.	17	135	122	134	106	134	128
,, aur.	21	99	105	117	101	110	110
Width, front. min.	9	114	101	101	116	103	—
,, supraorb. max.	—	120	110	108	126	108	—
,, bistephanic	10	120	118	125	106	120	(108)
,, front. max.	—	131	126	113	128	124	118
,, biauricular	11	139	—	131	—	138	—
,, occipit. max.	12	128	126	110	—	130	122
,, bimastoid, base	—	140	140	142	—	142	—
,, ,, apex	—	126	120	113	—	120	—

	Martin No.	S.W.	E.S.	F.	W.	K.	Swan.
Base-length (n.-b.)	5	113	(103)	100	86	107	(103)
For. magnum, l.	7	38	37	38	—	42	40
,, ,, w.	16	33	—	29·5	—	32	30
Bizygomatic, w.	—	146	135	132	134	140	—
Orbit, height	—	—	—	—	—	37	—
Frontal arc	26	132	126	132	128	138	(130)
,, chord	29	114	106	105	109	114	(111)
,, subtense	—	—	25	31	27	33	(29)
Parietal arc	27	123	116	116	116	116	116
,, chord	30	119	107	111	110	108	107
,, subtense	—	—	14	17	19	19	16
Occipital arc	28	119	126	142	119	123	116
,, chord	31	97	103	116	92	100	97
,, subtense	—	—	32	36	32	32	30
,, arc, supra-in.	—	75	80	97	74	77	64
,, ,, infra-in.	—	44	46	45	45	46	52
Coronal arc	24*b*	293	—	315	—	302	(304)
Ceph. index	8 : 1	78·8	80·6	79·3	83·8	79·4	(77·8)
Height, b.b. / Max. width	17 : 8	87·1	81·5	88·1	67·9	87·0	(87·6)
Cranial capacity	—	1357	1270	1478	1230	1358	1350

Martin. No. of measurement in Martin's *Lehrbuch*. 2nd ed.
S.W. Sir Arthur Smith Woodward. Measurements given by Friederichs from the reconstruction of 1922.
E.S. Sir Grafton Elliot Smith's reconstruction, 1927.
F. Dr F. Friederichs (1932).
W. Prof. Hans Weinert (1933).
K. Sir Arthur Keith (1938).
Swan. Measurements made on casts.

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OBSERVATIONS ON THE EFFERENT CONNEXIONS OF THE CENTRE MEDIAN NUCLEUS

By W. E. Le GROS CLARK and W. RITCHIE RUSSELL
Department of Human Anatomy, Oxford

In his classic work on the nervous system published in 1865, Luys described a number of centres in the thalamus of the human brain, which he was able to define anatomically. One of these was his *centre median* or "middle centre" which, as he indicated, occupies the middle of the thalamus on either side and, for the matter of that, approximately the central point of each cerebral hemisphere. For this reason, the nucleus might well be named the nucleus centralis of the thalamus except for the fact that this term has already been applied to other cell collections. As a matter of convenience, therefore, the nomenclature of Luys is commonly retained in the anglicized form of the *centre median nucleus*.

The centre median nucleus is most clearly defined as a separate element in the thalamus of higher mammals, and apparently reaches its highest differentiation in the human brain. It is well marked in apes and monkeys, and also in the larger lemurs. In lower forms it is often indistinct, and, as Rioch (1931) has shown, although it is probably present in all mammals, it may be indistinguishably blended with the nucleus parafascicularis. From comparative anatomical studies, it has been suggested that, since the nucleus appears to develop as an elaboration of intralamellar groups of cells strewn along the medial medullary lamina of the thalamus, it may be concerned with purely intrathalamic functions rather than with cortical projection. It was later found that cortical lesions in the monkey involving the frontal, parietal and temporal lobes were followed by no evident cell degeneration in the nucleus (Clark & Boggon, 1935), while after electrolytic lesions in the region of the nucleus in the cat, no fibres could be traced from it to the cortex by the Marchi method (Clark & Boggon, 1933). Finally, Walker's report (1938) on the degeneration in the thalamus of the monkey after hemi-decortication seems to make it certain that the centre median nucleus sends no fibres to the cortex. However, there still remains some doubt on this question, and this doubt was recently accentuated in the minds of the present writers by finding complete cell degeneration of the centre median nucleus in a human brain associated with a small circumscribed lesion in the external capsule, suggesting rather strongly that the nucleus may, after all, project on to the insular cortex. It may be noted, in this connexion, that the studies of Waller on the retrograde degeneration in the thalamus following cortical lesions in the rat (1934) and cat (1938) have led him to infer that the centre median nucleus does project on to the cortex. Here, however, we are confronted with difficulties of homologization, for the identification of the centre median nucleus in these lower mammals is a matter of considerable uncertainty.

In the present communication, a brief description is given of the human brain to which reference was made above, together with an account of the degeneration in the thalamus of a monkey in which the insular cortex had been completely destroyed by the method of devascularization.

DESCRIPTION OF THE HUMAN BRAIN

The brain was that of a woman who showed complete cortical deafness without aphasia as the result of bilateral thrombotic lesions. The clinical aspect of the case has been described elsewhere (Clark & Russell, 1938).

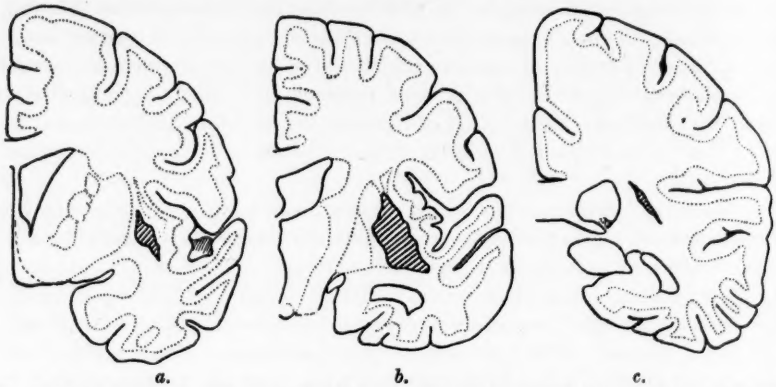

Fig. 1. Transverse sections through the right cerebral hemisphere of the human brain at the level of (*a*) the anterior extremity, (*b*) the middle, and (*c*) the posterior extremity of the lesion in the external capsule. Note that the lesion involves only the ventro-lateral part of the middle of the putamen. The caudate nucleus, the globus pallidus, and the greater part of the putamen remain intact.

Examined post mortem, the lesions were represented by a bilaterally symmetrical cavitation of the external capsule. On each side the cavity corresponds almost exactly in its extent with the overlying cortex of the insula. Rostrally (Text-fig. 1*a*), it is limited to the white matter of the ventral half of the external capsule between the lentiform nucleus and the claustrum. In the middle of its extent (Text-fig. 1*b*) it is broader, involving the greater part of the claustrum except for its dorsal extremity and the ventro-lateral part of the putamen of the lentiform nucleus. At its caudal extremity (Text-fig. 1*c*), which corresponds closely in level with the caudal limit of the insula, the lesion is again confined to the white matter. From the position of the lesion as shown on macroscopical examination, it is clear that practically the whole of the insular cortex had been isolated from the region of the internal capsule by the interruption of projection fibres. The fibres of the auditory radiations which pass to the upper surface of the middle third of the superior temporal gyrus had also been interrupted. Lastly, the dorsal margin of the lesion reaches a position where it is likely to have interrupted some of the projection fibres related to the lower extremities of the central convolutions.

Owing to the damage done to the thalamus on the left side of the brain by a pontine haemorrhage which occurred just before death, histological studies were confined to the right side. Sections through the site of the lesion were prepared by the Weigert-Pal method, and others were stained with toluidin blue. Serial transverse sections were also made through the caudal half of the thalamus and stained with toluidin blue in order to study the cellular degeneration related to the lesion in the external capsule.

The Weigert-Pal sections confirm the inferences drawn from the macroscopic appearance of the lesion in regard to the involvement of projection fibre systems and the limited involvement of the putamen. The serial sections through the thalamus show the following features. The medial geniculate body is completely degenerated; its cells have all disappeared and are replaced by a conspicuous gliosis. Cell degeneration has also occurred in the small intergeniculate nucleus (between the medial and lateral geniculate bodies), in the most ventral portion of the pars externa and pars arcuata of the ventral nucleus, and to a limited extent in the lateral and central areas of the pulvinar. On the other hand, the cytoarchitecture of the posterior projecting pole of the pulvinar is normal, and this is also the case with the lateral nucleus of the thalamus, the large medial (dorsomedial) nucleus, the parafascicular nucleus, and the greater part of the ventral nucleus (Text-fig. 2).

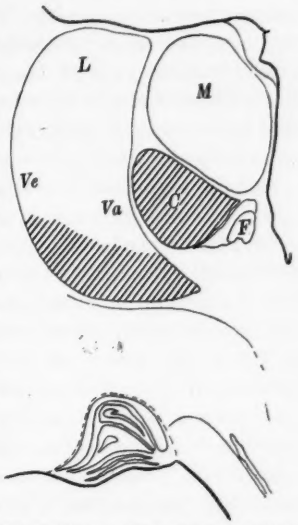

Fig. 2. Tracing of a transverse section through the posterior part of the thalamus of the human brain, showing the areas of total cell degeneration (marked by oblique lines). $\times 2\frac{1}{2}$. *C*, centre median nucleus; *F*, fasciculus retroflexus; *L*, lateral nucleus; *M*, medial (dorsomedial) nucleus; *Va*, pars arcuata of ventral nucleus; *Ve*, pars externa of ventral nucleus.

From the point of view of the cortical connexions of the thalamus, the most interesting observation is the almost total degeneration of the centre median nucleus. Its cells have completely disappeared, except for an insignificant number at its medial extremity, and the space which it normally occupies is marked out by an area of gliosis sharply circumscribed above and laterally by normal cells of the dorsomedial and ventral nuclei and medially by the parafascicular nucleus (Plate I).

DISCUSSION

From the description given above, it will be observed that on the right side of the brain the lesion was confined to the region of the external capsule. A close study of sections shows that it had interrupted fibres connecting with

the upper surface of the superior temporal gyrus (approximately the middle third), with the lower extremity of the central convolutions, and with the whole of the insular cortex, and, in addition, some of the fibres passing through the caudal end of the internal capsule to the parietal areas of the cortex were probably involved. Lastly, the middle portion of the lesion had involved the ventro-lateral angle of the putamen. Rostrally, however, the whole of the cross-sectional area of the lentiform nucleus was intact. It is therefore apparent that only a fraction of the whole putamen of the lentiform nucleus had been damaged. In the thalamus, serial sections show clearly marked areas of cell degeneration in the medio-ventral part of the ventral nucleus, in the whole of the medial geniculate body, and to a limited extent in the lateral nucleus and the pulvinar. These changes are readily explained by the involvement of the central convolutions, the auditory cortex, and fibres passing through the caudal extremity of the internal capsule. It remains to assign some cause to the degeneration of the centre median nucleus which is practically complete. There seem to be only two possible sources for this degeneration—the isolation of the insular cortex or the local damage to the putamen of the lentiform nucleus. We may first consider the latter possibility.

Papez & Rundles (1937), on the basis of observations on Weigert-Pal sections of normal human brains, inferred that the centre median nucleus projects on to the striatum, and they quote Auer & McCough (1916) who found extensive degeneration of the cells of the nucleus in a case of paralysis agitans in which there were numerous lesions in the corpus striatum. Walker found complete degeneration of the nucleus in a monkey after hemidecortication with marked damage to the basal ganglia, and, since in other cases in which the caudate nucleus and putamen had been partly destroyed the nucleus remained normal, he suggests that it probably projects on to the globus pallidus portion of the basal ganglia. In the case of the human brain here described, only a fraction of the putamen has been directly involved by the thrombotic lesion, nor is the latter situated in a position to interrupt possible thalamo-striate connections elsewhere. Certainly the globus pallidus seems to be eliminated from consideration, since the lesion had not damaged this element. If, therefore, the almost complete degeneration of the centre median nucleus is secondary to the damage of the basal ganglia, it must be supposed that the nucleus as a whole projects on to a very limited part of the putamen. This seems improbable, if only because such an interpretation is hardly consonant with the observations of comparative anatomy. The centre median nucleus becomes relatively larger and better defined in the higher primates, but this is not associated with any corresponding elaboration or cyto-architectural differentiation of the putamen of the lentiform nucleus.

The possibility of a projection of the centre median nucleus on to the island of Reil requires to be taken into account in observations based on lesions of the basal ganglia, since these will necessarily interrupt such projection fibres in their course to the cortex. Certainly, Walker seems to have eliminated this

possibility by his detailed studies of thalamic degeneration following hemidecortication in monkeys, although in his second case, in which the centre median nucleus stood out in particularly clear definition as a circumscribed mass of normal cells amid areas of total cell atrophy, a large portion of the island of Reil was reported to have been left untouched by the lesion.

In view of the apparent uncertainty regarding the relation of the centre median nucleus to the basal ganglia or to the overlying insular cortex, it seemed desirable to study the effect of a lesion involving the island of Reil in a monkey. At the same time, a preparation of this kind was required in order to determine whether the insular cortex receives projection fibres from any other nuclear element of the thalamus. In order to avoid any complicating factor which might be introduced by damage to the lentiform nucleus, a method had to be devised whereby the whole thickness of the insular cortex could be completely destroyed without affecting the basal ganglia. As they are separated by a thin layer of white matter (the external capsule) which in some places is not more than 1 mm. wide, the usual technique of cortical ablation could hardly be employed. It was decided, therefore, to destroy the insular cortex by devascularization, a method which would leave the lentiform nucleus intact, since this structure receives its blood supply from basal arteries, while the cortex is entirely supplied by pial vessels. The following is a description of the experiment.

Monkey 51*a*. Operation. Under nembutal anaesthesia, an osteoplastic flap was turned down low on the left side, exposing the whole length of the Sylvian sulcus. With an endothermy, the blood vessels all along the lips of the sulcus were coagulated. At its anterior extremity, the temporal pole was carefully separated from the lower margin of the frontal lobe so as to expose the limen insulae. Here the branches of the middle cerebral artery passing to the insula were brought to view and coagulated.

The operation was followed by a rapid recovery with no apparent motor defect.

Four weeks later, the animal was killed by ether anaesthesia and the brain removed. The site of the lesion was filled with a slough of necrotic tissue, the remains of the devascularized cortex. In its superficial extent, the lesion was confined to the immediate margin of the Sylvian sulcus and measured 29 mm. in length. It was limited caudo-ventrally by the parallel sulcus (Text-fig. 3). The brain was cut into transverse slices which showed that the lesion had involved the whole extent of the insular cortex together with the lips of the Sylvian sulcus, while the basal ganglia (exclusive of the claustrum) appeared to be intact. At its posterior extremity, the lesion had evidently involved little more than the actual grey matter of the cortex.

Transverse sections taken through the length of the lesion and stained with methylene blue showed that it was limited to the cortex and the immediately subjacent white matter (Text-fig. 4). The whole of the insular cortex had undergone complete necrosis, as well as the submerged cortex forming the lips

of the Sylvian sulcus. The lentiform nucleus was intact except possibly for a few superficial cells lying along the extreme lateral margin of the putamen. Anteriorly the dorsal part of the claustrum was uninjured, while the ventral

Fig. 3. Lateral view of brain of monkey 51*a*, showing the extent of the cortical lesion following devascularization. × 1.

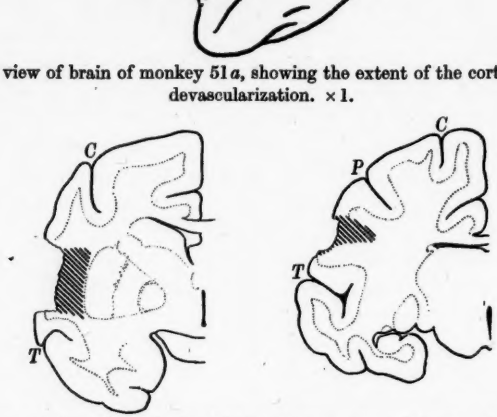

Fig. 4. Transverse sections through the right hemisphere of monkey 51*a*, showing the extent of the cortical lesion. × 1. *C*, central sulcus; *P*, intraparietal sulcus; *T*, superior temporal sulcus. Note that the lesion is confined to the cortex and immediately subjacent white matter.

part had been involved in the necrosis of the cortex. Posteriorly the lesion had involved only the central part of the claustrum. It is still a matter of argument whether the claustrum should be properly regarded as a cortical or striatal element. Its mode of development (Rose, 1928) and its blood supply (Shellshear, 1920) suggest that it belongs to both structures. This experiment provides additional evidence that the claustrum is only partly vascularized from pial vessels, the remainder being supplied from striate arteries. At its posterior extremity, the lesion is seen in the microscopic sections to be limited to the cortex of the Sylvian sulcus and an extremely thin layer of the subjacent white matter. It is apparent, indeed, that it has not involved deep-lying fibre tracts passing back to cortical areas behind the level of the lesion.

Serial transverse sections were taken through the thalamus and stained with methylene blue. These show well-defined circumscribed areas of cell atrophy and accompanying gliosis in the following places—the ventral margin

of the dorsomedial nucleus, the ventromedial region of the ventral nuclear mass, the medial part of the main element of the pulvinar (nucleus $p\beta$ of Friedemann) extending back as far as the level where the pulvinar forms a freely projecting pole, and the medial geniculate body. These areas of degeneration (as shown by previous experiments) correspond respectively to the involvement by the lesion of (1) the lower margin of the granular frontal cortex, (2) the lower extremities of the central convolutions, (3) the lips of the Sylvian sulcus behind the auditory cortex, and (4) the auditory cortex. There is no need to refer further to these points, except for a brief remark on the cell degeneration in the pulvinar. This corresponds to the area of atrophy which has been previously reported after lesions involving the lips of the Sylvian sulcus immediately behind the auditory cortex (Clark, 1936). Whereas, however, the previous conclusions may be open to criticism on the ground that the cortical ablation had injured subjacent fibre paths connecting the pulvinar with more caudally situated regions of the cortex, in the present experiment this probably does not apply, since the method of devascularization has led to a minimal involvement of the white matter in immediate relation to the necrosed cortex.

The centre median nucleus is apparently quite intact. If there is any cell degeneration in this thalamic element, it is too slight and diffuse to be detected. It may therefore be concluded that in the rhesus monkey the centre median nucleus probably does not project at all on to the insular cortex, nor (as previous experiments have repeatedly shown) on to any other cortical area. On the other hand, no cell degeneration in other thalamic elements could be attributed to the insular lesion, and it may be inferred, therefore, that the insular cortex of the monkey does not receive any thalamic projections. In the absence of such connexions, the insular cortex contrasts with probably all other cortical areas in the monkey with the exception of the greater part of the temporal lobe.

GENERAL DISCUSSION

The results of the experimental lesion in the monkey do not bear out the inference drawn from the human brain described in this paper that the centre median nucleus may project on to the insular cortex. In connexion with this, the following considerations require to be taken into account.

It is possible that the centre median nucleus may project on to the insular cortex in man although it has no such connexions in the monkey. Rose (1929) has shown that in the human brain the insular cortex is characterized by an extensive differentiation of the granular fields, and by the appearance of a well-defined area (subregio tenuigranularis) such as is not to be found in lower primates. He also delineated four separate granular fields in the insular cortex of the lemur, twenty in the monkey, and as many as thirty-one in man. This elaboration of the insular cortex in the human brain, including the differentiation of a new field not represented in lower mammals, may be referable to the development of a projection system from the centre median nucleus. On the

other hand, the latter (as compared with the homologous nucleus in the monkey) does not show histologically any differentiation in cyto-architecture which might be expected if this were the case.

If the degeneration of the centre median nucleus described in the human brain is not the result of the interruption of fibre connexions with the insular cortex, it is presumably secondary to the partial destruction of the putamen of the lentiform nucleus, and this would indicate that the centre median nucleus projects on to a limited portion of the putamen. This conclusion is in accord with Walker's suggestion that the nucleus is connected with the basal ganglia, except that it seems to eliminate the globus pallidus from consideration in this regard. On the other hand, Walker reports experiments on the monkey in which there occurred partial damage of the caudate nucleus and putamen without degeneration in the centre median nucleus. Thus the question of the extrathalamic efferent connexions of the centre median nucleus still remains unsettled.

SUMMARY

A human brain is described in which a lesion in the external capsule, isolating the insular cortex and involving a small portion of the putamen, is associated with almost complete degeneration of the centre median nucleus. The possibility of a cortical projection from the nucleus to the insular cortex was tested by the experimental destruction of the insular cortex in a monkey by devascularization. The results of this experiment provide no evidence that the island of Reil in the monkey receives projection fibres from the centre median nucleus, or from any other nuclear element of the thalamus.

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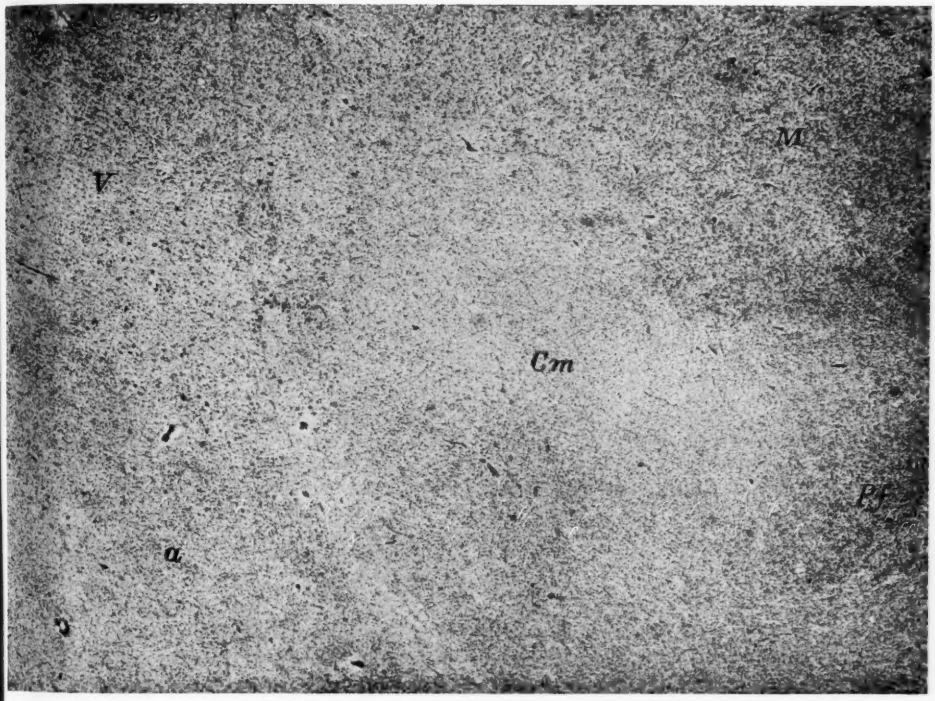
EXPLANATION OF PLATE I

Transverse section through right side of thalamus of the human brain showing the circumscribed area of degeneration in the centre median nucleus. × 15. The normal cells of the medial (dorsomedial) nucleus and the ventral nucleus can be seen above and to the lateral side. The area of cell degeneration in the ventral extremity of the ventral nucleus (*a*) can also be seen. *Cm*, centre median nucleus; *Pf*, parafascicular nucleus; *M*, medial (dorsomedial) nucleus; *V*, ventral nucleus.



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NOTES ON THE COMPARATIVE ANATOMY OF THE GANGLION CELLS ASSOCIATED WITH THE VERTEBRATE INNER EAR SENSORY AREAS

By JEAN K. WESTON[1]

From the Anatomical Institute of the University of Groningen, Holland

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INTRODUCTION

The shifting of nerve cells, both phylogenetically and ontogenetically, is a phenomenon which early attracted the attention of neuroanatomists. The study of these shiftings eventually resulted in Ariëns Kappers's (1914, and earlier) principle of neurobiotaxis—a far-reaching generalization that has proved of great value and importance in explaining many otherwise seemingly anomalous and unrelated morphological data. Such shifting of nerve cells has been thoroughly substantiated, the movement apparently being toward their centre of greatest stimulation. The ganglion cells associated with the VIIIth cranial nerve are good examples of such shifting, even embryologically (Streeter, 1907); this is likewise true of their central nuclei of termination (Ariëns Kappers, 1934).

Almost any diagrammatic illustration of the nerve supply of the inner ear which pretends to accuracy will serve as an excellent example of such shifting of nerve cells. Such a diagram is Fig. 1, where the cells of the cochlear (spiral; Corti's) ganglion are represented as lying near to the sensory epithelium with which they are concerned (organ of Corti), both actually and relatively, while the cells of the vestibular (Scarpa's) ganglion lie at a noticeably greater distance from their sensory epithelium, both actually and relatively. Furthermore, the ganglion cells of the ganglion spirale lie at a considerably greater distance from

[1] This study was accomplished while the author was on leave for a year from the Laboratory of Comparative Neurology, University of Michigan, Ann Arbor, Michigan.

the central nervous system than do those of the vestibular ganglion. Such relations, clearly indicative of a differential shifting, become even more noticeable if photographic illustrations are examined.

The sensory areas of the inner ear have been classed into two general types, perilymphatic and endolymphatic (de Burlet, 1935), depending upon whether they lie in relation with a direct (unobstructed by connective tissue strands) perilymphatic channel or not. The truly perilymphatic type areas (Fig. 2) are the papilla amphibiorum and the papilla basilaris (organ of Corti); at times quasi-perilymphatic claims are made by the macula lagenae and the macula sacculi. The truly perilymphatic sensory areas are further characterized by the possession of a tectorial membrane and (excepting among the amphibians) a basilar membrane. The above facts all suggest a hearing function for such perilymphatic areas (and thus they may be called cochlear type areas), which is supported by the available experimental evidence, at least on the higher forms. The endolymphatic areas (firmly attached to the otic capsule by connective tissue strands ramifying through the surrounding perilymph) consist of the crista anterior, crista externa, crista posterior, macula utriculi, and papilla neglecta (Fig. 2). These have all been shown experimentally (excepting the papilla neglecta) to possess an equilibratory function. The macula sacculi and the macula lagenae, while frequently seemingly more endolymphatic in type, do not clearly fall into either class at all times from a structural standpoint, and, while in fishes these two have been shown to possess a hearing function (von Frisch, 1938), as yet no equilibratory function has been definitely and unquestionably established for them. It is interesting to note that the truly perilymphatic areas are always found in relation to the pars posterior of the developing labyrinth (pars inferior of the adult), as are the somewhat indeterminate areas (macula lagenae and macula sacculi)—excepting for that part of the macula sacculi innervated by the superior saccular ramus of Voit (1907); the certainly endolymphatic areas are related to both the pars anterior and the pars posterior of the developing labyrinth (pars superior of the adult). For more details relative to the various methods of subdividing the labyrinth, see de Burlet (1931); also Fig. 2.

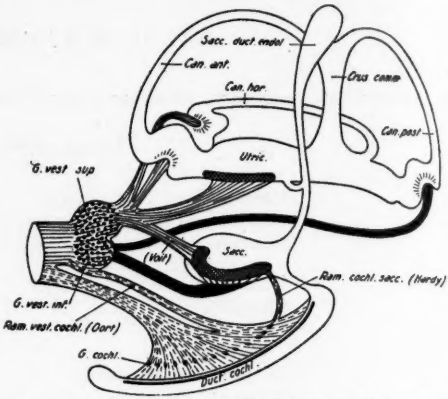

Fig. 1. This figure is a generalized diagram of the mammalian labyrinth, illustrating especially the relations of the various nerve branches to the sensory areas. The legends are obvious. Note especially the branches reported by Voit (1907), Oort (1918), and Hardy (1934).

Attention has previously been called to the close proximity of the ganglion cells of the ganglion spirale to the organ of Corti in mammals and some Sauropsida (de Burlet, 1934; Weston, 1937; see also Ariëns Kappers, 1934), although in a rather general way. Further comparative study of the inner ear

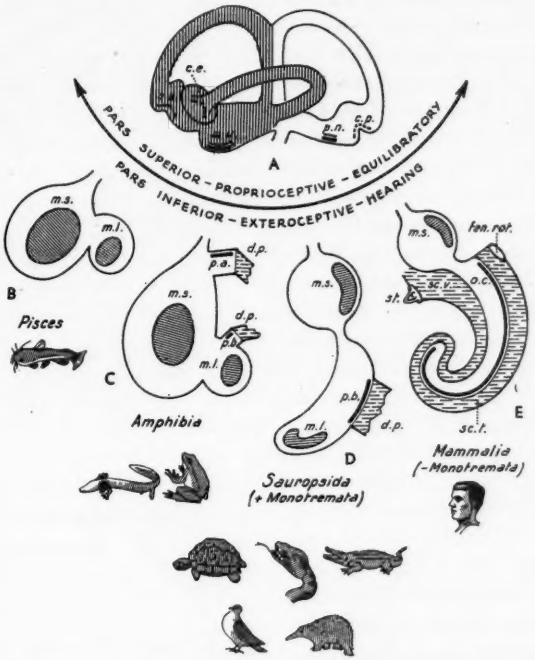

Fig. 2. Diagrams of the vertebrate labyrinth. A, pars superior (not including Cyclostomata and Holocephali). *c.a.* crista anterior; *c.e.* crista externa; *c.p.* crista posterior; *p.n.* papilla neglecta; *m.u.* macula utriculi. B, C, D, E, pars inferior (classes and orders of vertebrates as indicated). *m.l.* macula lagenae; *m.s.* macula sacculi; *p.a.* papilla amphibiorum; *p.b.* papilla basilaris; *o.c.* organ of Corti; *d.p.* ductus perilymphaticus; *fen.rot.* fenestra rotundum; *sc.v.* scala vestibuli; *sc.t.* scala tympani; *st.* stapes. The sensory areas included within the shaded portion of A are those which are always supplied by the ramus anterior of the VIIIth nerve; when Voit's anastomosis is present a portion of the macula sacculi is also supplied by the ramus anterior.

has raised the question as to whether all the truly perilymphatic areas demonstrate this same relationship to some degree, in more or less distinct contrast to the endolymphatic areas. If true, this would be further very suggestive evidence favouring their possession of a common (hearing) function wherever such truly perilymphatic areas exist. It would likewise suggest that the factors responsible for the presence or absence of the direct perilymphatic channel are intimately related to those responsible for the differential shifting of the associated ganglion cells.

Although the exact reason for this differential shifting of the ganglion cells associated with the inner ear sensory areas is not known, Fig. 3 summarizes what is known in that connexion. Fig. 3A shows the various areas (all originally ectodermal in derivation) which may conceivably (and which various authors affirm) give rise to the ganglion cells associated with the VIIIth nerve. Fig. 3B illustrates the older concept of the origin of the ganglion cells associated with the cranial (and spinal) nerves from the neural crest, and their subsequent wandering along their dendrite toward the site of their greatest stimulation, in accordance with the principle of neurobiotaxis. Fig. 3C gives the result of a similar type of reasoning with regard to the more modern embryological results, particularly those of Niessing (1932), van Campenhout (1935), and Yntema (1937), who have produced evidence to the effect that the ganglion cells associated with the VIIIth nerve are largely derived from the otic vesicle. This being true, they might have migrated centralward differentially as shown. However, it is known that this primordial migration from the otic vesicle occurs very early in embryological development. Furthermore, the work of Streeter (1907), on human embryos, has demonstrated that, in development, there is actually a (secondary) migration of the cells of the ganglion spirale from a position near the ganglion vestibulare to their final resting place in the adult. These facts are harmonized in Fig. 3D, which is more likely what has actually occurred—first a migration centrally[1] from the otic vesicle to the

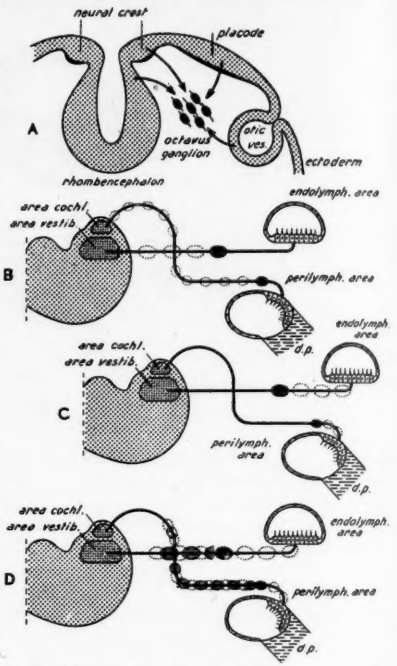

Fig. 3. Schematic representation of the origin and possible means of migration of the ganglion cells associated with the two types of inner ear sensory areas. The text sufficiently clarifies this figure. The legends are obvious.

[1] Not knowing certainly the source, the nature, or the duration of the attractive influence, if any, which would be operative, it is more difficult to explain this *first* migration by applying the relatively simple notion of neurobiotactic movement toward the centre of greatest stimulation. Perhaps this primary migration is rather to be explained by what Ariëns Kappers (1934) refers to as "mnemic predecession", possibly even harking back phylogenetically to the invertebrate condition and the probable withdrawal of neurosensory cells from the surface to form primitive ganglion cells (see Ariëns Kappers, 1929, pp. 5–7, 14). However, this phenomenon may also be in line with the observation that neuroblasts may shift with the current (stimuloconcurrently) so long as they have not yet developed Nissl substance (Ariëns Kappers, 1929, p. 12).

same degree, regardless of the future fate or function of the migrating cells, and a subsequent, differential, peripheralward migration, perhaps as the result of the neurobiotactic attraction of the functioning end-organ. This still leaves wide open the question as to why the hearing end-organ should have a stronger affinity for its associated ganglion cells than the equilibratory end-organs. The only attempt at an explanation of this point known to the author is a suggestion of Ariëns Kappers (1934). This observer, proceeding from the viewpoint that the cochlear end-organ is more gnostic or epicritic in character and that the equilibratory end-organs are more protopathic in character, suggested that the difference in threshold value of these two types of stimuli was the factor responsible for the position of their associated ganglion cells, the more epicritic (hearing) end-organ (organ of Corti), having the lower threshold and hence being more readily and frequently stimulated, exerting the stronger attractive force upon its associated ganglion cells. He also finds attributive evidence for this in the somewhat parallel central migration of the primary cochlear centres phylogenetically.

No doubt partly dependent upon the above differential migration, and certainly of no less importance (probably more important from a practical standpoint), is the precise localization within the VIIIth nerve ganglia of the cells having to do with each of the sensory areas of the inner ear. Little attention appears to have been paid to this important matter, judging from the literature, the work of Beccari (1912, on the lizard) and of Lorente de No (1933, and earlier, on the mouse and cat) being the main contributions to this subject.

The purpose of this study is, then, to determine, so far as the material permits, the positions of the ganglion cells related to each of the various inner ear sensory areas (a matter that seems to have been somewhat neglected), and to examine particularly the relationships of the ganglion cells associated with the perilymphatic, as compared with those associated with the endolymphatic, sensory areas of the inner ear throughout vertebrates.

MATERIALS AND METHODS

Numerous series of sections of the inner ears of various members of all vertebrate classes were consulted. For the most part, these were stained with hematoxylin and eosin, and frequently sectioned in both frontal and sagittal planes. All the material examined was obtained from the large collection of the Anatomical Institute of the University of Groningen.[1]

The species consulted most in the preparation of this report were the following: Mammalia: *Echidna*, *Talpa*, *Erinaceus*, bat, *Cavia*, *Sciurus*, *Mus*,

[1] Since this will doubtless be one of the last products of a year's study at the Anatomical Institute of the University of Groningen, the author takes this opportunity of expressing his very real gratitude to Prof. H. M. de Burlet, Director of the Institute, for the many things he has done in a variety of ways to make this year both pleasant and productive. Sincere thanks are also due to all the members of the Institute for many kindnesses and much assistance; especially to O. Kerssen for his careful and skilful drawing of numerous illustrations, both for publication and otherwise.

Lepus, Felis, Canis, Macacus, Homo; Aves: *Tyto, Turdus, Taenio, Munia, Passer, Columba*; Reptilia: *Sphenodon, Chelhydra, Emys, Testudo, Tropidonotus, Bungaris, Xenopeltis, Homalopsis, Dendrolophis, Coluber, Naja, Calotes, Adder, Agama, Anguis, Seps, Chamaesaura, Gecko, Lygosoma, Mabya, Lacerta, Pachydactylus, Uromastix, Iguana, Chameleon, Crocodilus*; Amphibia: *Ichthyophis, Proteus, Salamandra, Triton, Axolotl, Bombinator, Rana, Bufo*; Pisces: *Petromyzon, Mustelus, Acanthias, Protopterus, Chimaera, Hippocampus, Clarius, Amiurus, Anguilla, Pleuronectes, Syngnathus, Xiphophorus.*

OBSERVATIONS

In the following descriptions, the ganglion cell position will be characterized either as intracranial or intraotic. Intracranial ganglion cells, for the purposes of this study, are considered to be those lying definitely within the cranial cavity or within any of its more or less confluent depressions or meatuses. Intraotic ganglion cells are those definitely completely included within the otic capsule or caught within the bony or cartilaginous wall of that capsule. It is realized that the above definitions are somewhat arbitrary, but they can be qualified when necessary. Some attention will also be devoted to the relative sizes of the ganglion cells, as well as to their relative distances from the end-organs with which they are associated.

Mammalia

The mammalian condition, so far as the inner ear ganglion cells are concerned, is well illustrated in Fig. 4, a photomicrograph of the inner ear of a dog (see also Figs. 1 and 2). From this photograph it is obvious that the ganglion cells associated with the vestibular nerve are almost entirely intracranial in position, tending to be grouped into two masses, the superior and inferior vestibular ganglia, which are more or less confluent at their medial extremities (see also Alexander, 1899). However, it must be noted that all mammals do not demonstrate such a definite separation into superior and inferior vestibular ganglia, for in certain forms (e.g. *Talpa*) there is no hint of such a division, and here one may only speak of superior and inferior portions of the vestibular ganglion.

The superior vestibular ganglion supplies fibres to the crista anterior, crista externa, macula utriculi, and, excepting in *Echidna*, the more anterior and superior portion of the macula sacculi (via the ramus saccularis superior of Voit, 1907). The inferior vestibular ganglion supplies fibres chiefly to the crista posterior and the balance of the macula sacculi, with the exception of that portion of the latter supplied by the small group of intraotic ganglion cells (more vestibular in type) noted within the bony meshes near the inferiormost portion of the macula sacculi (for a more detailed consideration of such ganglion cells, see Weston, 1937). There is also a considerable probability that either or both the vestibular ganglia give rise to the fibres of Oort's anastomosis *a*, and

that the inferior vestibular ganglion contributes to Oort's anastomosis *b*. With the possible exception of those ganglion cells of the vestibular ganglia which may be associated with Oort's anastomoses, all the cochlear ganglion cells are intraotic in position, actually lying within and filling, to a greater or less degree, the canalis spiralis modioli, as Wolff (1936) has noted for man and some other mammals. In *Echidna* (see Weston, 1937, Figs. 8 and 9) and certain other

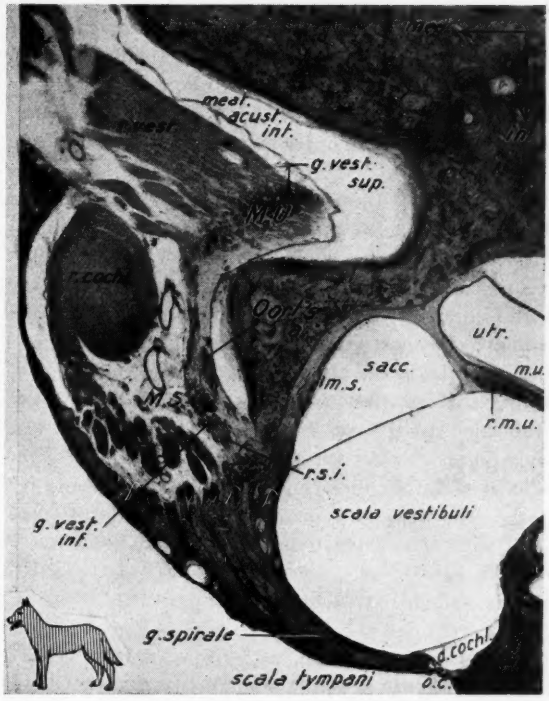

Fig. 4. Frontal section through the left ear of a dog. ×20. Note especially the positions of the ganglion cells. This figure is sufficiently explained in the text. The labels are obvious. *r.s.i.* ramus saccularis inferior.

mammals (e.g. *Talpa*), the cochlear ganglion cells lie largely entirely within the bony otic capsule rather than within a well-formed canalis spiralis modioli.

Relative to the localization of the sensory areas within the ganglia, for the superior vestibular ganglion it appears that the ganglion cells associated with the crista anterior are found most rostrodorsally, followed in order caudoventrally by those for the crista externa, macula utriculi, and (when a ramus saccularis superior is present) the anterior portion of the macula sacculi.

A concentration of noticeably larger cells is observed in the ganglion cell areas associated with the two cristae, although many medium sized cells are also present which are apparently similar to those noted within the ganglion cell area devoted to the macula utriculi and the macula sacculi. In spite of the definiteness of such a general localization, the material suggests the strong possibility of a certain amount of intermingling between the fibres coming from these sensory areas; there even appear to be a few fibres passing between the superior and inferior vestibular ganglia. It was noticeable that when the intraotic relations were such that the nerve coming from the crista externa was forced to loop somewhat ventrally below the macula utriculi, its ganglion cells nevertheless retained their more dorsal position, even though that frequently necessitated such crista externa fibres threading through certain of the fibres passing to the macula utriculi in order to reach its more dorsally lying ganglion cells.

Within the inferior vestibular ganglion and lying more anteriorly and nearer the bony wall adjacent to the macula sacculi were the ganglion cells concerned with most of the macula sacculi exclusive of the anterior portion of that macula; in size, these resembled the ganglion cells associated with the macula utriculi (see Fig. 4). The intraotic ganglion cells related to the macula sacculi appeared to be most concerned with the most inferior and posterior portion of that macula, as did the ramus cochleo-saccularis, regardless of the possible origin of this last (for more details see Hardy, 1934; Weston, 1937). Occupying the same relative position, but at more caudal levels, and lying nearer the nervous cochlearis throughout the more rostral portion of their extent, were located the ganglion cells interested in the crista posterior. Here, too, many large cells were concentrated. The exact position of the ganglion cells associated with Oort's anastomoses could not be definitely located (see Weston, 1937).

No attempt is made here to determine the projection of the various portions of the organ of Corti upon the ganglion spirale. For those interested in this matter, reference is made to the investigations of Held (1926), Cajal (1928), Guild *et al.* (1931), Lorente de No (1933), Wolff (1936).

The above ganglion cell localization is illustrated in Fig. 5, so far as the relation to the otic capsule and the relative dorsoventral position of the cells are concerned, although this figure cannot stress the fact that there is a rostrocaudal difference in position as well. This localization agrees essentially with that reported by Lorente de No (1933, and earlier) for the mouse and the cat. The absence of a distinct inferior vestibular ganglion in certain forms simply means that the ganglion cells have remained in one mass, and apparently results in no noticeable difference in the *relative* localization of the sensory areas within that mass.

So far as the relative distance of the ganglion cells from their respective sensory areas is concerned, it is obvious from Figs. 1, 4 and 5 that those ganglion cells associated with the organ of Corti lie nearest that strip of sensory epi-

thelium (although those intraotic ganglion cells associated with the more posterior and inferior portion of the macula sacculi also lie very near that area; Weston, 1937). However, it must be remembered in this connexion that there is as yet no general agreement that the area most adjacent to any cochlear ganglion cells is always the area to which those cells distribute; furthermore, it appears that there are forms where the cochlear ganglion cells extend only about halfway up the modiolar axis (Wolff, 1936). The ganglion cells which lie next closest to their sensory areas appear to be those concerned with the macula utriculi and macula sacculi; the ganglion cells lying farthest from their associated sensory areas are those concerned with the three cristae.

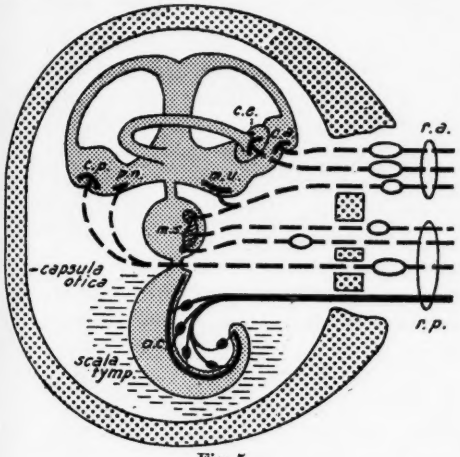

Fig. 5.

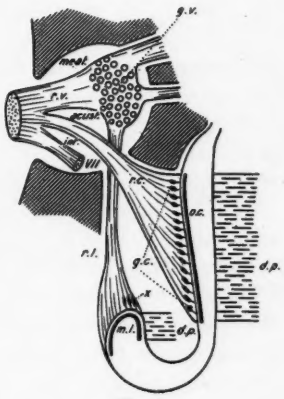

Fig. 6.

Fig. 5. A generalized and schematic diagram of the location of the ganglion cells related to the various inner ear sensory areas of mammals. See Fig. 2 for the meanings of the abbreviations used.

Fig. 6. A schematic diagram of the perilymphatic and ganglion cell relations of the organ of Corti and macula lagenae of *Echidna*, slightly modified from de Burlet. Most of the abbreviations are clarified in Fig. 2. *x*, ganglion cells associated with only the perilymphatic portion of the macula lagenae.

Since the inner ear of *Echnida* is somewhat unique among mammals, it deserves a little more comment. Some of its chief points of interest (see also Fig. 6) from the standpoint of this study are: (1) the lack of coiling of the cochlea; (2) the possession of a macula lagenae; (3) the absence of the ramus saccularis superior of Voit; (4) the lack of a distinct canalis spiralis modioli; (5) the extraordinarily large size of the macula utriculi (60 % of the total inner ear sensory area). It is of especial interest to note that the macula lagenae of *Echnida* is partly in relation with a direct perilymphatic channel. Even more striking with relation to this study is the fact that the ganglion cells associated with the perilymphatic portion of the macula lagenae

are intraotic in position and lie very near that portion, while those associated with the more endolymphatic portion of the macula lagenae lie within the vestibular ganglion and are intracranial in position, and as far distant from their end-organ as are those associated with any of the cristae. This certainly makes it appear that there may well be an intimate correlation between the presence of a direct perilymphatic channel and the proximity of the ganglion cells (and possibly even the function), so far as these are related to the inner ear sensory areas.

Aves

The ganglion cell characteristics of birds are exceptionally well shown in Fig. 7A. Clearly, there is no division here into a superior and an inferior vestibular ganglion. The ganglion cells occupying an intraotic position in this microphotograph are for the supply of but two of the inner ear sensory areas, the organ of Corti and (in part) the macula lagenae; all the other sensory areas are supplied by the intracranially lying ganglion cells. These last ganglion cells lie in a rather shallow fossa instead of in the deep meatus observed among most mammals (Fig. 7A).

The localization of the various sensory areas within this ganglion cell mass was even more definite than in mammals, but was not radically different from the mammalian condition. The nerve branch coming from the crista anterior could clearly be followed as a distinct bundle (even having a separate exit through the bone, see Fig. 7B) into the more rostrodorsal portion of the intracranially lying cells, rostral to the level of Fig. 7A. As Fig. 7A shows fairly well, the fibres from the crista externa enter the ganglion more caudoventrally than do those from the crista anterior, followed in turn by the fibres from the macula utriculi. At more caudal levels than Fig. 7A, the ganglion cells related to the macula sacculi appear first, followed by those related to the crista posterior and a few which appear to be associated with the macula lagenae. The localization in birds is more of a dorsoventral one than in mammals, the ganglionic mass apparently being somewhat more compact in birds. Such a dorsoventral localization, as well as the relation of the ganglion cells to the otic capsule is diagrammatically illustrated in Fig. 7B. Within the otic capsule, the more medially and dorsally lying cells are associated with the macula lagenae; the others (by far the most numerous) with the organ of Corti.

Here, too, the differences in size of the ganglion cells making up the above localized areas is particularly obvious from Fig. 7A. The definitely large cells in the figure are associated with the crista anterior and externa; similar cells are numerous in the area associated with the crista posterior. The more medium sized ganglion cells send their dendrites to the macula utriculi, the macula sacculi (not showing in Fig. 7A), and the macula lagenae (lying mostly intraotically); the smallest intraotically lying ganglion cells, as Fig. 7A demonstrates, are concerned with the organ of Corti. The larger (medium sized) intraotically lying cells noted in the same figure are for the supply of the macula lagenae.

Again, as in mammals, the ganglion cells associated with the three cristae lie farthest from their end-organs, although the ganglion cells interested in the macula lagenae are very likely little, if any nearer to that end-organ, in spite of their predominant intraotic position. The ganglion cells related to the organ of

Fig. 7 A.

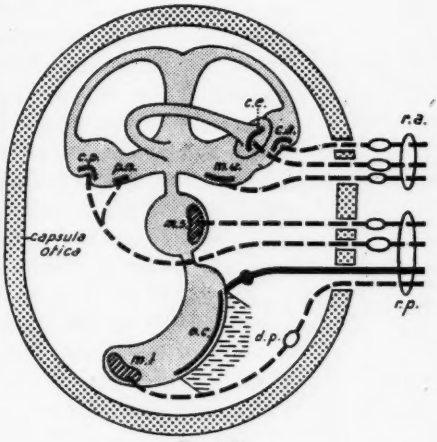

Fig. 7 B.

Fig. 7. A, frontal section through the inner ear of the dove. ×35. Note especially the positions and sizes of the ganglion cells. B, a generalized and schematic diagram of the location of the ganglion cells related to the various inner ear sensory areas of birds. Fig. 2 will clarify most of the abbreviations used. The capital letters, i.e. *C.A.* indicate the ganglion cell position where that sensory area, i.e. crista anterior, is projected. *r.c.* ramus cochlearis; *r.c.a.* ramus crista anterior; *r.c.e.* ramus crista externa; *r.m.u.* ramus macula utriculi; *r.v.* ramus vestibularis.

Corti lie nearest that end-organ. Those related to the macula sacculi (and, in large part, the macula lagenae) are somewhat intermediate as regards their distance from those sensory areas. In certain cases it appeared that the ganglion cells concerned with the macula sacculi lie relatively quite near that area,

although remaining intracranial, but such relationships seemed to be somewhat fortuitous and certainly inconstant.

In brief, one may say that the birds resemble *Echidna* very much in all respects, with the exception of the innervation of the macula sacculi. In birds there is but one branch to the macula sacculi, and no trace of the so-called cochleo-saccular ramus (Hardy, 1934; Weston, 1937) is present. Neither were there any intraotic ganglion cells concerned with the innervation of the macula sacculi and the crista posterior (see Fig. 8), which, in birds, were entirely intracranial. It is notable, too, that there was more of a tendency for the ganglion cells related to the different sensory areas supplied by the ramus posterior to be somewhat

Reptilia

In view of a previous study of the central connexions of the vestibular nerve among reptiles (Weston, 1936), this class was of particular interest. In the following account, a general notion of the reptilian characteristics will first be laid down. Following that, attention will be given to the individual variations and points of interest observed among the different members of this vertebrate class.

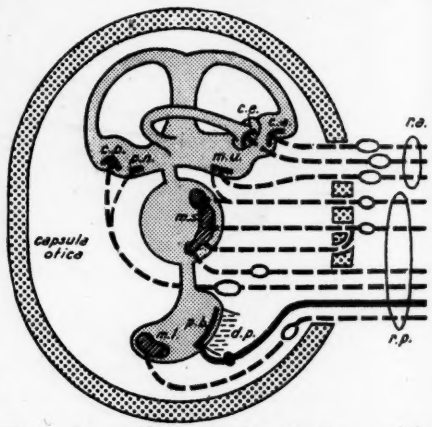

Fig. 8. A generalized and schematic diagram of the location of the ganglion cells related to the various inner ear sensory areas of reptiles, based particularly on the condition observed in *Sphenodon*. Fig. 2 sufficiently explains the abbreviations.

In general, the ganglion cells associated with the ramus anterior (which supplies the crista anterior, crista externa, macula utriculi, and sometimes the upper portion of the macula sacculi; see de Burlet, 1929) occupy an intracranial position among reptiles, while the ganglion cells associated with the ramus posterior (which supplies the crista posterior, papilla neglecta, macula lagenae, a portion, at least, of the macula sacculi, and the papilla basilaris (organ of Corti), tend to be largely intraotic, the most usual exceptions being those ganglion cells concerned in part with the macula sacculi, the macula lagenae, and the crista posterior (and papilla neglecta). This is diagrammaticaly illustrated in Fig. 8, which represents the condition in *Sphenodon*; Figs. 9 and 10 also illustrate certain of these facts.

The localization of the various inner ear sensory areas upon the ganglion cells was strictly comparable to the condition noted in birds, with the exception

separated from each other spatially, rather than to lie so closely adjacent as in birds. This localization is well shown in Figs. 8, 9 and 10.

The general size variation found in birds and mammals appears to be the rule among reptiles also, that is, largest cells related to the cristae, medium sized cells chiefly to the maculae, and smallest cells to the organ of Corti or papilla basilaris (see Fig. 8). However, this size difference was much less obvious in some reptiles than in others, especially as between the cells concerned with the maculae and those related to the papilla basilaris or organ of Corti (see Figs. 9 and 10).

With regard to the relative proximity of the ganglion cells to their sensory areas, the usual condition is that the cristae lie farthest (excepting crista posterior in some cases), the macula lagenae next farthest (possibly farther than the crista in some cases), the macula utriculi and the macula sacculi next in order, and the papilla basilaris (organ of Corti) nearest their ganglion cells (see Figs. 8, 9 and 10). Although in some cases the papilla basilaris did not appear to be much, if any, nearer its ganglion cells than portions of the macula lagenae and macula sacculi, yet in practically no case did it lie farther from those cells than either of the other two. Likewise, this close relationship was more consistently exhibited in the case of the ganglion cells related to the papilla basilaris than that of the macula sacculi or macula lagenae when the various associated maculae lay at a considerable distance from the main ganglion cell mass.

One of the chief differences between the crocodilian and avian condition is the presence of intraotic ganglion cells in crocodiles which supply the inferior (and posterior) portion of the macula sacculi. Such cells are well shown in Fig. 10D and have not been noted at all among birds. Although the sizes and staining reactions of these cells do not vary markedly from those concerned with the organ of Corti, their affinity is unmistakable in the sections. They would appear to be homologous to those ganglion cells found within the meshes of bone in the dog (see Fig. 4; also Weston, 1937). In addition to these, the same figure shows a small strand of fibres, apparently definitely coming from a cell region clearly concerned with the organ of Corti, which also pass to the more inferior portion of the macula sacculi. These last appear to be definitely the homologue of the ramus cochleo-saccularis of Hardy (1934; Fig. 1), and would agree well with her description of that ramus in man as coming from the region of cochlear ganglion cells, although it could not be definitely observed in the sections whether the fibres originated from cochlear ganglion cells or otherwise (see Weston, 1937). Although most of the ganglion cells supplying the crista posterior and the inferior (and posterior) portion of the macula sacculi were intraotic in position, a small number supplying these sensory areas were also intracranial.

The lizards resembled the crocodile very much in most respects. Fig. 9A illustrates the significant relations relative to the ramus anterior. Figs. 10A and B make clear the relative ganglion cell positions as these apply to the

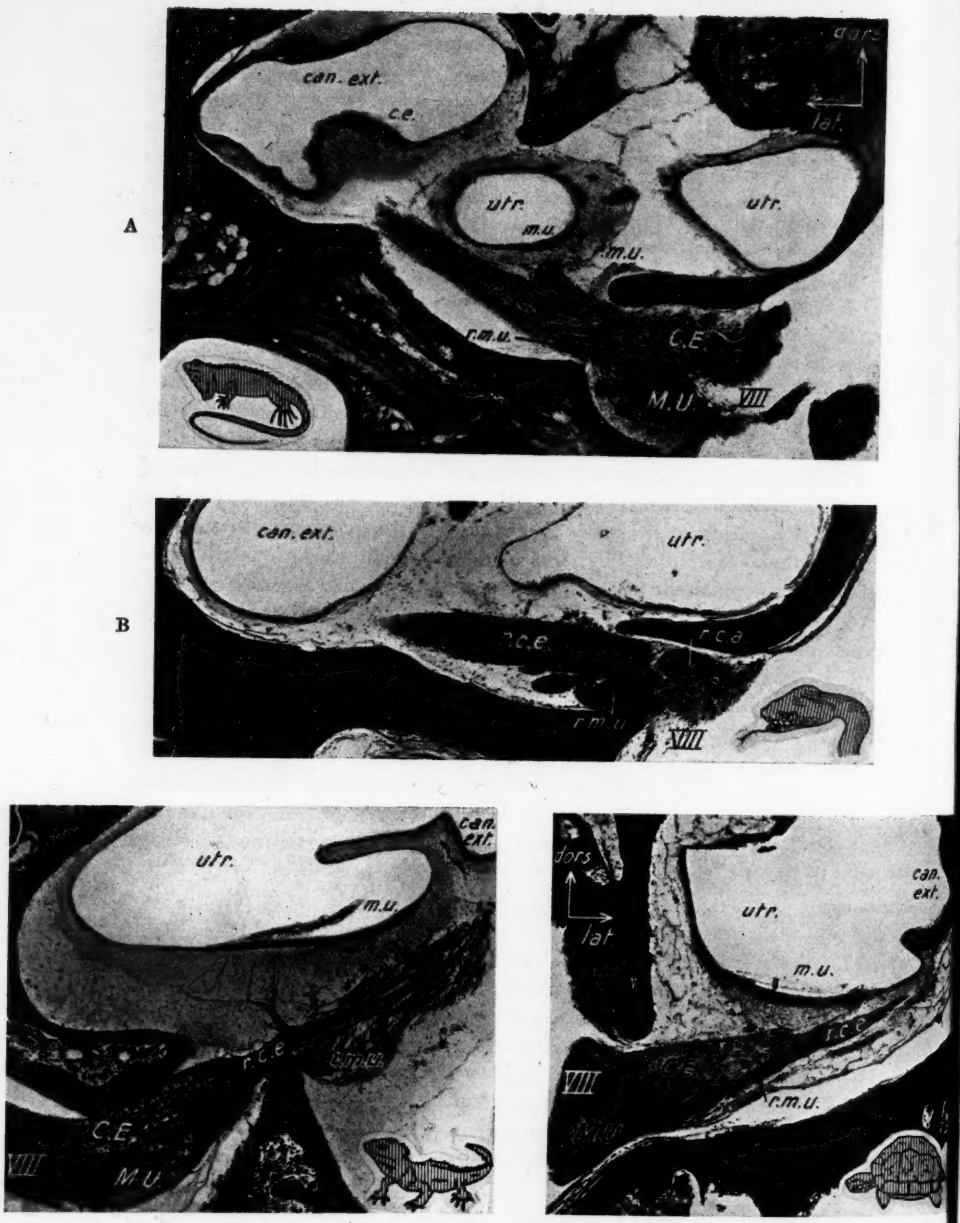
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Fig. 9.

greater portion of the ramus posterior. No clearly obvious cochleo-saccular ramus could be established among any of the lizards. In some of the lizards (e.g. *Chameleon*) all the ganglion cells related to the macula sacculi were intracranial in position. Although as a rule smaller, the ganglion cells associated with the papilla basilaris were not much different in size from those associated with the macula lagenae and macula sacculi in some lizards (see Fig. 10). In many of the lizards the intracranially lying ganglion cells associated with the macula sacculi lay very near that sensory area, but it appeared that this was a rather inconstant relationship. The Gecko was one of the very few cases encountered where some of the ganglion cells associated with the ramus anterior occupied an intraotic position, and here the brain case seemed to be fully occupied by the brain itself. In several cases (e.g. *Lygosoma*), the ganglion cells concerned with the macula lagenae were intracranial in large part.

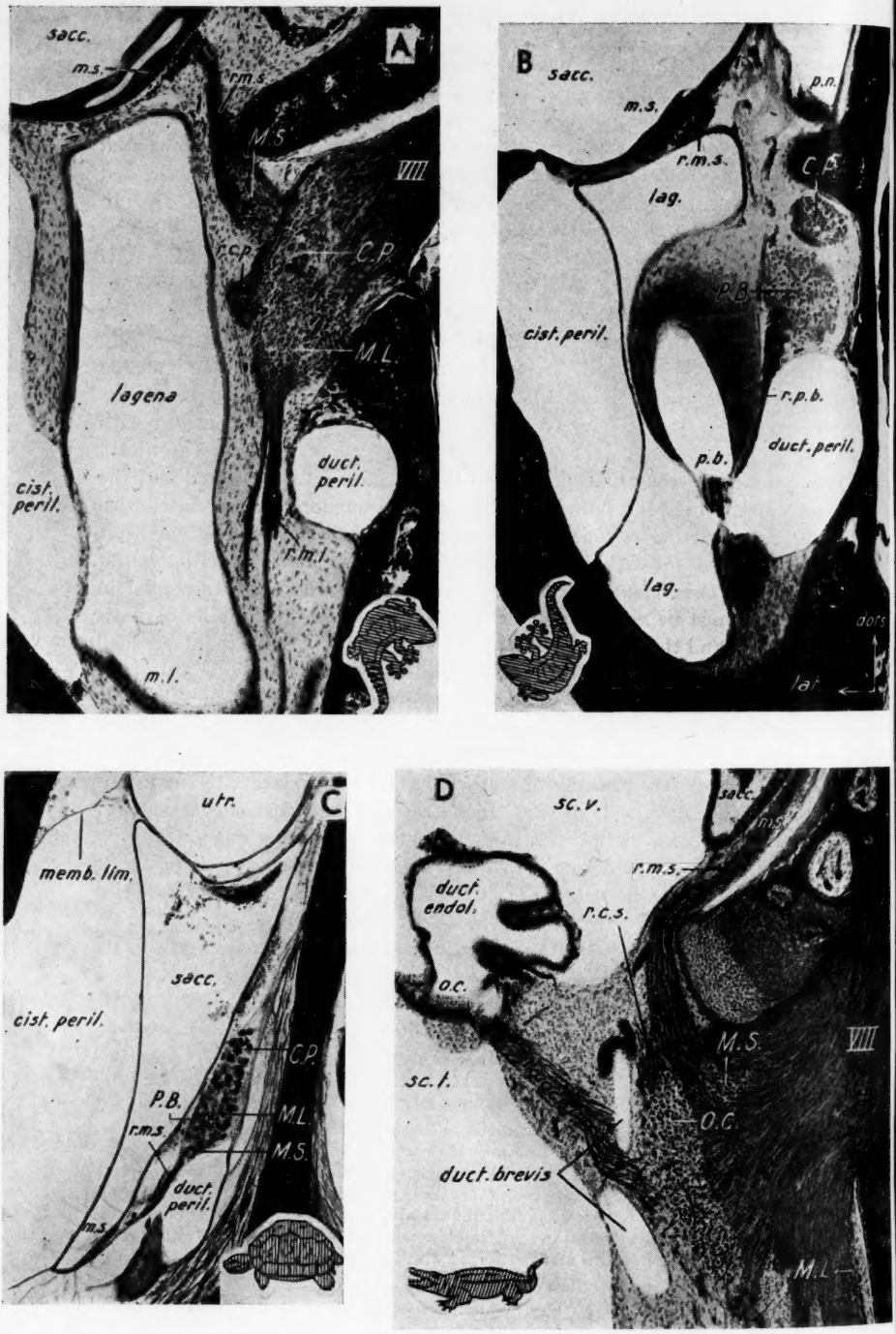
The snakes resemble closely all the other forms so far considered with respect to the ganglion cells associated with the ramus anterior (Fig. 9B). There appears a strong tendency for all the ganglion cells associated with the ramus posterior to lie intracranially, although the ganglion cells associated with the papilla basilaris, especially, and with the crista posterior, seem most reluctant to give up their intraotic position. This tendency finds its fullest expression in *Dendrolophis*, where all the ganglion cells are intracranial in position. It must be noted, however, that among snakes the papilla basilaris is very small, and that even when its associated ganglion cells are intracranial in position, they still usually lie closer to that sensory area than do most of the other ganglion cells to their respective end-organs. The statements made above for the lizards relative to the position of the macula sacculi ganglion cells and the sizes of the various ganglion cells apply equally well to snakes.

The turtles closely resemble the other reptiles with relation to the ramus anterior ganglion cells (see Fig. 9D). In one series of *Testudo*, however, some of the ganglion cells associated with that ramus appeared to lie intraotically, as the figure shows. Most of the ganglion cells related to the ramus posterior occupy an intraotic position (see Fig. 10C). Those related to the crista posterior seem to have migrated exceptionally far toward that crista in some cases.

Sphenodon is quite similar to the turtles regarding its ganglion cells. Fig. 9C illustrates the condition with respect to the ramus anterior. Fig. 8 is a diagram of the relations found in *Sphenodon*, and this diagram shows but little variation from the generalized reptilian condition. One rather unusual variation, not only from the condition in other reptiles, but from any form observed

LEGEND TO FIG. 9

Fig. 9. Microphotographs illustrating the ganglion cell position in relation to the ramus anterior of the VIIIth nerve. The abbreviations are sufficiently clarified in Figs. 1 and 7. A, iguana. ×35. Note the obvious crossing of the fibres coming from the macula utriculi and from the crista externa. B, *Tropidonotus*. ×50. C, *Sphenodon*. ×35. D, *Testudo*. ×35. Note the obvious crossing of the fibres coming from the macula utriculi to reach their ventrally lying ganglion cells. Note also that here a considerable number of the ganglion cells lie intraotically. Observe the larger cells in the areas concerned with the cristae, especially in A, C and D.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duct. endol.
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sc. l.
O.C.
duct. brevis
M.L.

Fig. 10.

or reported in the literature, is the presence of a small bundle of fibres which is associated with the macula utriculi but leaves the otic capsule with the branch concerned with the more superior portion of the macula sacculi—a Voit's anastomosis in reverse, so to speak. This relationship was bilateral. In another available series of *Sphenodon* it also appeared to occur, although in this last series it could not be so easily or clearly followed, due to the broken condition of the sections in this particular region.

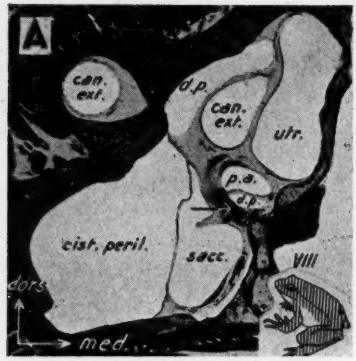
Amphibia

The amphibians, particularly the tailless ones, do not exhibit radical differences from the generalized reptilian condition so far as their ganglion cells are concerned. The ganglion cells associated with the ramus anterior are always intracranial in position; those associated with the ramus posterior are mixed, some being intracranial and others intraotic. The tailed amphibians demonstrate a greater proportion of the ganglion cells associated with the ramus posterior lying intracranially than do the tailless amphibians.

Although no sections were encountered which demonstrated it as plainly as can be seen in Fig. 9, for reptiles, serial sections make it clear that the ganglion cells concerned with the crista anterior, crista externa, macula utriculi and macula sacculi in tailless amphibians (e.g. *Bufo asper*) lie intracranially and in the same relative position as noted for reptiles. Although not quite so obvious, this appears equally true for the tailed amphibians, excepting for the most inferior and posterior portion of the macula sacculi, which in certain of these (*Salamandra*, for example) is supplied by intraotically lying ganglion cells. Caudal to the ganglion cells associated with the macula sacculi are found the ganglion cells concerned with the papilla amphibiorum (in *Bufo asper*, at least); these are practically entirely intraotic regardless of the amphibian consulted (see Fig. 11E), although particularly numerous in *Bufo asper*, in consequence of the very large papilla amphibiorum present in this toad. Immediately inferior and slightly caudal to these are noted the ganglion cells (also intraotic) concerned with the papilla basilaris (Fig. 11E). Lying largely, if not entirely, intracranially and somewhat mediocaudal and inferior to the foregoing (and at times somewhat intraotic in position) are found the ganglion cells concerned with the crista posterior and the macula lagenae (see Figs. 11B, F). In some cases (e.g. *Bombinator*, Fig. 11D) ganglion cells related to the macula sacculi occupy a similar position. It was notable that the ganglion cell localization here was more in the rostrocaudal than in the dorso-

LEGEND TO FIG. 10.

Fig. 10. Microphotographs illustrating the ganglion cell position in relation to the ramus posterior of the VIIIth nerve. The abbreviations may be clarified by reference to Figs. 2 and 7. A, Gecko. ×70. B, Gecko. ×50. B is more caudal than A. Note the ganglion cells related to the more inferior and caudal portion of the macula sacculi. C, *Testudo*. ×30. Note especially the nerve supply to the caudal and inferior portion of the macula sacculi. D, *Crocodilus*. ×30. Observe the ramus cochleo-saccularis (*r.c.s.*) and the positions of the other ganglion cells,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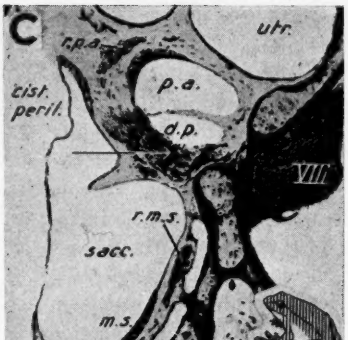
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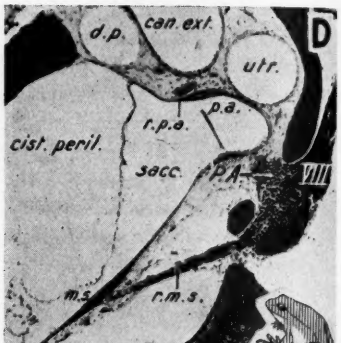
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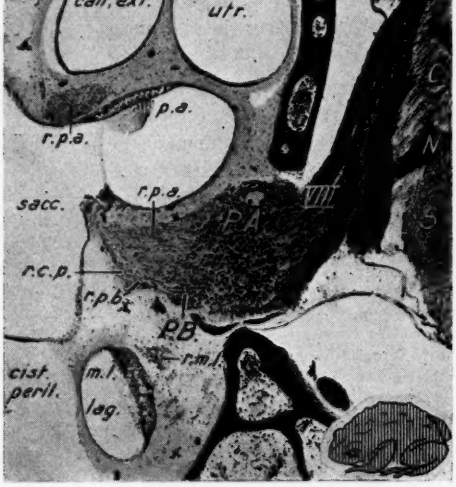
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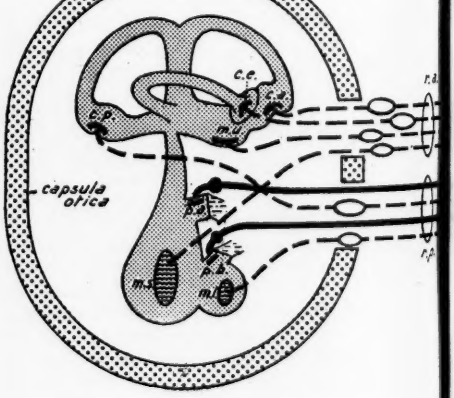
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ventral plane, and that there was not much tendency for the ganglion cells to move far from the main clump associated with the ramus anterior and the ramus posterior of the VIIIth nerve.

With relation to the size of the cells, those concerned with the cristae are partly of large size, while those concerned with the papilla basilaris are apparently uniformly small (Fig. 11 E). The ones related to the maculae are more medium sized. As Fig. 11 E shows, the cells concerned with the papilla amphibiorum are more nearly medium sized than like those related to the papilla basilaris.

The ganglion cells concerned with the papilla basilaris and the papilla amphibiorum lie closest to those end-organs in nearly every case. However, there are some amphibians where it appears that some of the ganglion cells associated with the macula lagenae, especially (and also with the macula sacculi), are almost equally as near to that end-organ. Yet when the intraotic relations of either the macula lagenae or the macula sacculi are such that they are farther from the main ganglionic mass concerned with the VIIIth nerve (as in *Bombinator pachypus*, Fig. 11 D), their associated cells make no effort to move intracranially to them. The ganglion cells related to the maculae are next farthest from those end-organs; those concerned with the cristae lie farthest from those end-organs.

Although the foregoing statements are based mainly upon the condition in the Anura, they are also generally true for the tailed amphibians (with the previously noted reservation that not so many ganglion cells occupy an intraotic position), although not so clearly or easily established there because of the seemingly somewhat smaller number of cells and the fact that they appear more clumped together.

It is noteworthy that in amphibians, the only class where the papilla amphibiorum (a truly perilymphatic sensory area) occurs—a sense organ which has not yet been definitely homologized with any inner ear structure of other vertebrates (see de Burlet, 1928)—the ganglion cells concerned with the innervation of that area exhibit a marked and unquestionable affinity for it (Fig. 11 E). When this fact is coupled with the presence of a tectorial membrane (see Fig. 11 B, D, E; also Retzius, 1881; Gaupp, 1904; Larsell, 1934), the presence of a direct perilymphatic channel in association with it (de Burlet,

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LEGEND TO FIG. 11.

Fig. 11. Microphotographs illustrating the ganglion cell position in relation to the ramus posterior of the VIIIth nerve. A and B, *Bombinator igneus*. × 50. C, *Bombinator igneus*, A enlarged to × 70. Note the relation of the ganglion cells to the papilla amphibiorum and the macula lagenae. D, *Bombinator pachypus*. × 50. Compare the relation of the ganglion cells associated with the macula sacculi with those associated with the papilla amphibiorum in C or E. E, *Bufo asper*. × 70. Note the ganglion cells associated with the papilla basilaris. F, a generalized and schematic diagram of the location of the ganglion cells related to the various inner ear sensory areas of amphibians, based especially on *Rana* and *Bufo*. Note the position of the ganglion cells related to the perilymphatic areas. Figs. 2 and 7 clarify the abbreviations used.

1929), and the fact that this area is apparently concerned with the more dorsal medullar centres (see Fig. 11 E) whose central connexions are more cochlear in type (Larsell, 1934), added emphasis is given the statement of Larsell (1934) that these "appear sufficient to indicate that the papilla serves an acoustic function, and that its fibres are not vestibular". It is also interesting, in this connexion, that there are amphibians with no papilla basilaris (*Proteus*) and that in all cases the papilla amphibiorum is much larger than the papilla basilaris.

It should be observed that the diagram (Fig. 11 F) is based upon the condition noted for *Bufo asper* and *Rana* and is not strictly applicable to all the amphibians, either urodele or Anura, so far as the exits of the fibres associated with the macula sacculi and the macula lagenae are concerned (see Figs. 11 B, D).

Pisces

It is not easy to make definite statements relative to the intracranial or intraotic position of the ganglion cells in fishes because of the fact that the medial wall of the otic capsule fails over a large area in many forms (*Petromyzon*, *Anguilla*, siluroids). Likewise, perhaps correlated with this failure, the inner ear itself is much crowded and in many cases lies closely adjacent to the brain (especially in siluroids). However, in practically all cases where one could speak of an otic capsule as distinct from the cranial cavity, the ganglion cells associated with the ramus anterior occupied their usual intracranial position. The ganglion cells associated with the ramus posterior were intracranial for the most part, but those associated particularly with the crista posterior demonstrated a tendency to migrate within the otic capsule. These facts are diagrammatically shown in Fig. 12 G. It should be noted that in one fish examined, *Chimaera monstrosa*, all the ganglion cells were intraotic in position.

Although the above-noted anatomical variations naturally exert some influence, yet it is clear in the fishes, even in *Petromyzon*, that the ganglion cells associated with the crista anterior and crista externa (where present) lie rostrodorsally of those concerned with the macula utriculi (see Figs. 12 A, B, C, D and G). This means that the ganglion cells concerned with the pars anterior sensory areas have changed little in relative position from *Petromyzon* to man. Usually the ganglion cells related to the macula sacculi lie most inferiorly and

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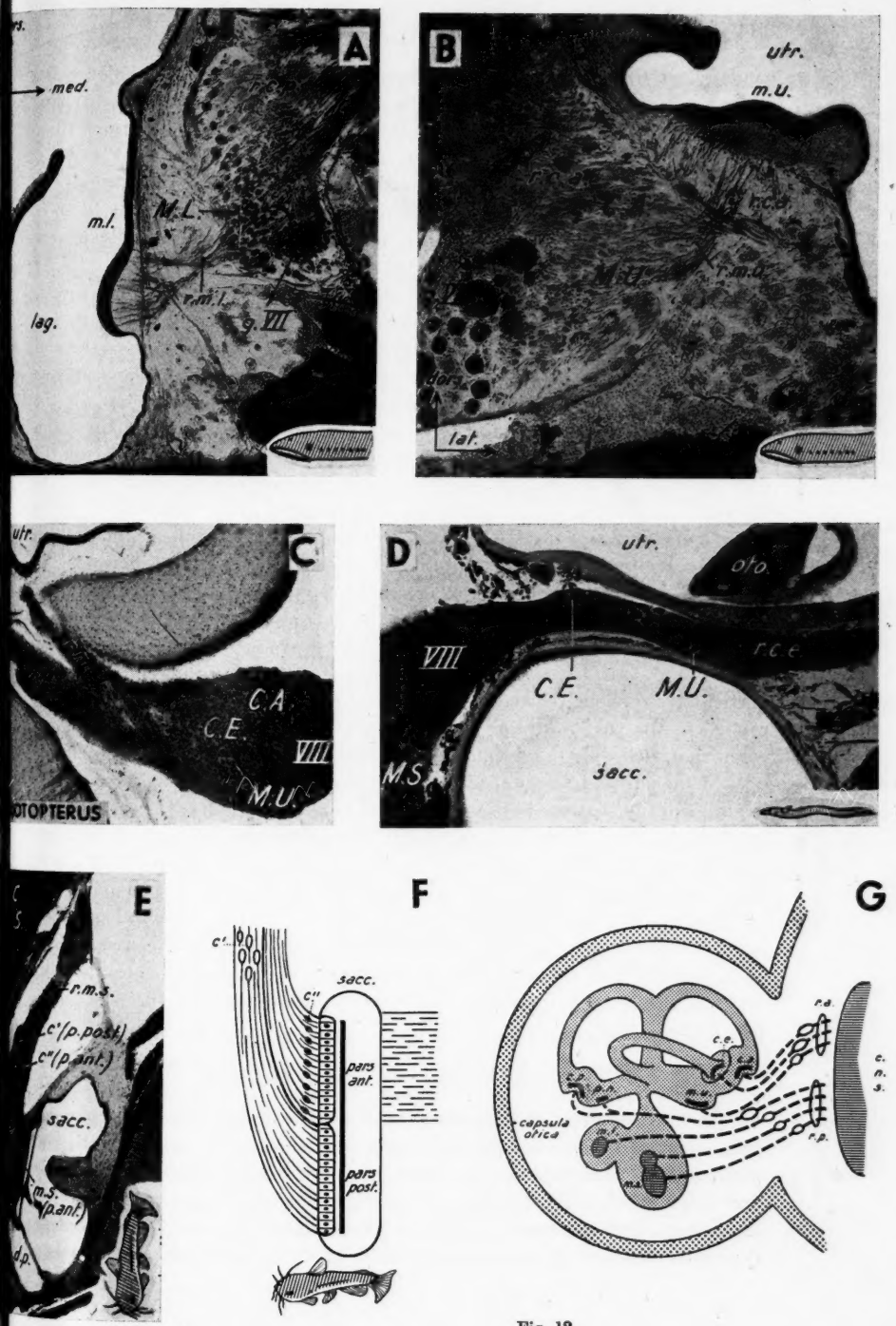
LEGEND TO FIG. 12.

Fig. 12. Microphotographs illustrating the position of the ganglion cells in relation to the VIIIth nerve among fishes. A and B, *Petromyzon*. × 80. Note the size of both fibres and cells related to the cristae as compared with those related to the other sensory areas. C, *Protopterus*. × 30. Note again how the fibres from the macula utriculi seek ventrally lying ganglion cells. D, *Anguilla*. × 70. Note the differences in size and position of the ganglion cells. E, *Amiurus*. × 55. Actual photograph of ganglion cells related to the macula sacculi of *Amiurus*. F, a highly schematized diagram of the ganglion cell positions relative to the macula sacculi of *Amiurus*. G, a generalized and schematic diagram of the location of the ganglion cells related to the various inner ear sensory areas of fishes. Figs. 2 and 7 will clarify all abbreviations used.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G
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capsula otica
r.p.

Fig. 12.

caudally, at times even completely separated from the other ganglion cells, while those concerned with the crista posterior (and papilla neglecta) and macula lagena lie more rostrodorsal, but frequently completely separated from the ganglion cells related to the ramus anterior (see Figs. 12D, E, F and G).

Due to the variability in the relationships between the otic capsule and the brain case, generalizations as to the proximity of the ganglion cells to their end-organs are impossible. In various forms the macula utriculi may lie nearest its associated end-organ (Fig. 12D); in others, the macula sacculi (Fig. 12E, F) or macula lagenae is closest; in still others, the crista posterior is nearest. Of especial interest in this connexion was the condition observed in the siluroids examined, *Amiurus* and *Clarius*. The fish, *Amiurus*, has been demonstrated by von Frisch (1938, and earlier) to be capable of hearing nearly as well as man in some respects. Earlier, de Burlet (1929) had noted for the siluroid fishes, of which *Amiurus* is one, certain relationships of the swim bladder to the perilymphatic system which appeared to him to confer on the pars anterior of the macula sacculi a close relationship to what might be likened to a direct perilymphatic channel as found in other vertebrates. From these facts, it was considered probable that eventually a hearing function might be related to this pars anterior of the macula sacculi in siluroids. In Fig. 12F, although somewhat exaggerated, is shown diagrammatically the position of the ganglion cells related to the pars anterior and those related to the pars posterior of the macula sacculi, as these are shown in the microphotograph in Fig. 12E, for *Amiurus*. It must be noted that size differences were not particularly obvious between the cells related to the pars anterior and those related to the pars posterior of the macula sacculi. It is interesting, in this connexion, that only in the siluroid fishes has any high order of hearing ability been demonstrated to date, although it has not yet been possible to prove experimentally that the pars anterior of the macula sacculi is the sensory area responsible. Yet, from an anatomical viewpoint, the relation of this pars anterior area to a direct perilymphatic channel and the relative proximity of its associated ganglion cells are strongly suggestive that it is the responsible agent.

The largest ganglion cells associated with the VIIIth nerve in fishes were again located in the area from which dendrites distribute to the cristae (Figs. 12A, B and D); more medium sized ganglion cells supplied the other sensory areas. This was more noticeable in *Petromyzon* than in any of the other fishes (see Figs. 12A, B).

DISCUSSION AND CONCLUSIONS

The consistent intraotic position and relative proximity of the ganglion cells associated with the vertebrate papilla basilaris or organ of Corti, both perilymphatic type sensory areas, strongly suggest a correlation here which is probably linked with the type of function (vibratory perception) of these end-organs. This is the more striking when it is recalled that no endolymphatic type area consistently demonstrates either of such characteristics throughout

vertebrates. When the papilla amphibiorum, a well developed perilymphatic type end-organ found only among amphibians (de Burlet, 1928), demonstrates the same relations to its ganglion cells, the above notion is supported—and a function suggested for this structure which is similar to that possessed by the papilla basilaris and the organ of Corti. Perhaps nowhere is the effect of the presence of the direct perilymphatic channel upon the position of the associated ganglion cells more clearly shown than with relation to the macula lagena in *Echidna* (Fig. 6) and the macula sacculi in *Amiurus* (Fig. 12F), for in these animals the association of the direct perilymphatic channel with an inner ear sensory area appears clearly to exert an irresistible attraction for the ganglion cells concerned with such areas. Consequently, it appears safe to conclude that those inner ear sensory areas associated with a direct perilymphatic channel also tend to have their related ganglion cells placed within the otic capsule and relatively near to such sensory areas.

The localization within the VIIIth nerve ganglia of the ganglion cells related to the various inner ear sensory areas is apparently a rather simple and logical one. For the sensory areas supplied by the ramus anterior of the VIIIth nerve, the ganglion cells concerned with the crista anterior are most rostodorsal, followed in turn caudoventrally by the ganglion cells concerned with the crista externa, those supplying the macula utriculi, and, when a ramus saccularis superior of Voit is present, those related to the pars anterior of the macula sacculi (chiefly in mammals and reptiles). This ganglion cell localization in relation to the sensory areas supplied by the ramus anterior appears somewhat stereotyped throughout the vertebrates, from fishes to man. However, this is not especially surprising, for those sensory areas do not exhibit any radical variation throughout the vertebrates. This uniformity of the ganglion cell position suggests the strong probability of a similar uniformity in the fundamental plan of the central projection of such inner ear sensory areas.

The position of the ganglion cells in relation to the sensory areas supplied by the ramus posterior exhibits somewhat more variability, but this is perhaps largely the result of the different positional relations of these sensory areas. Usually the ganglion cells related to the macula sacculi lie somewhat caudoventral to those concerned with the macula utriculi, followed in order caudally (and ventrally) by the ganglion cells concerned with the crista posterior, papilla basilaris—organ of Corti (and papilla amphibiorum, when present), and macula lagenae. Usually the ganglion cells related to the papilla amphibiorum, papilla basilaris, or organ of Corti lie laterally (in forms below mammals) to those concerned with the macula lagenae; more dorsally (and sometimes laterally; sometimes medially) to the former are found the ganglion cells related to the crista posterior (and papilla neglecta). While all these ganglion cells related to the ramus posterior sensory areas are noted occupying an intraotic position to some extent, those concerned with the macula sacculi, crista posterior (and papilla neglecta), and macula lagenae are predominantly intracranial in position.

The variation in the nerve supply to the macula sacculi has been previously commented upon by de Burlet (1929), who considered such variation partially explainable by the difference in position of the macula sacculi throughout vertebrates, as well as the mode of separation and growth of the various sensory areas from the one primordial area. The ganglion cell relationship to the macula sacculi also exhibits considerable variation. However, for the most part, the associated ganglion cells lie intracranial in position, sometimes quite near the sensory area and at others at a considerable distance from it. Very commonly among mammals, reptiles, and some amphibians, the most inferior and posterior portion of the macula sacculi has a separate nerve bundle (de Burlet, 1929), and curiously enough, the associated ganglion cells usually approach this portion of the macula quite closely. This relationship of the ganglion cells does not always appear to be explainable by the position of the portion of the macula sacculi being discussed (see Figs. 10, 11, 12), and in view of the foregoing statements, suggests a function for that portion of the macula sacculi that is different from the remainder of that macula (see Weston, 1937). The relation of this bundle of fibres and its ganglion cells to the inferior and posterior portion of the macula sacculi to the so-called cochleo-saccular ramus reported by Hardy (1934; see also Weston, 1937) is not clear, since only in crocodiles (below mammals) was an apparent homologue of the cochleo-saccular ramus to be found (see Fig. 10D). Likewise, the wide separation of the nerve fibres and ganglion cells supplying the different portions of the macula sacculi, especially in reptiles, suggest the feasibility of future experimental attack upon the vexed problem of saccular function.

The rather general presence throughout vertebrates of large ganglion cells in the group supplying the three cristae, of medium sized ganglion cells in the group supplying the maculae, and of smaller ganglion cells in the group supplying the perilymphatic areas, appears to be correlated with the relative distances these various sensory areas lie from their ganglion cells. As observed, the cristae usually lie farthest from, and the perilymphatic type sensory areas (papilla basilaris—organ of Corti, papilla amphibiorum) nearest to their associated ganglion cells, with the maculae occupying more of an intermediate position. These facts will very probably prove to be eventually correlated with the various kinds of clearly recognizable action currents found within the vestibular nerve (see especially Ross, 1936).

Quite aside from the interest and value of such morphological details as have been brought out here, the establishment of the approximate positions of the ganglion cells related to each of the inner ear sensory areas, as has been done in the foregoing study, is a definite and necessary step forward in the direction of obtaining a knowledge of the more precise central representation of these various sensory areas, either by normal or by experimental anatomical study.

SUMMARY

1. The ganglion cells associated with the VIIIth nerve are partly intraotic and partly intracranial in position.

2. The intraotic portion is practically always related to the papilla basilaris (organ of Corti) and the papilla amphibiorum; frequently it is related to the crista posterior (and papilla neglecta), the macula lagenae, and the most inferior and posterior portion of the macula sacculi.

3. The intracranial portion is practically always related to the crista anterior, crista externa, macula utriculi, and most of the macula sacculi; it is usually related to the crista posterior (and papilla neglecta); it is sometimes related to the macula lagenae.

4. The ganglion cells related to perilymphatic or cochlear type sensory areas (papilla amphibiorum and papilla basilaris—organ of Corti) tend to lie consistently nearer such areas than do the ganglion cells related to the more endolymphatic type areas; the ganglion cells related to the cristae tend to lie farthest from such end-organs.

5. The ganglion cells vary from large to small: the large ones are associated with the cristae; those intermediate in size, with the maculae; the smaller ones with the papilla amphibiorum and papilla basilaris—organ of Corti.

6. The sensory areas are projected upon the ganglion cells so that the crista anterior is most rostrodorsal, followed in order (progressing from anterodorsal to caudoventral) by the crista externa, the macula utriculi, the macula sacculi, the crista posterior (and papilla neglecta), the macula lagenae, and the papilla basilaris (organ of Corti); the positions of the first three of the above areas within the ganglia are relatively constant throughout the vertebrates—the others are frequently subject to considerable variation.

7. A homologue of the ramus cochleo-saccularis, first described in man by Hardy (1934), appears to be present in crocodiles.

8. Sphenodon exhibited a communication from the macula utriculi which left the otic capsule with the most superior saccular ramus, apparently the reverse of the ramus of Voit (1907).

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THE FORMATION OF THE UMBILICAL CORD AND THE UMBILICAL REGION OF THE ANTERIOR ABDOMINAL WALL

By GEORGE M. WYBURN

Department of Anatomy, The University of Glasgow

INTRODUCTION

"It is a characteristic of many vertebrates that, associated with the provision of special arrangements for nourishing the young individual, the time of commencing an independent existence on its own account is greatly delayed. In such cases where a considerable proportion of the whole development takes place within the shelter of the egg shell or parental body we have to do with what is known as embryonic in contradistinction to larval development" (Graham Kerr).

The young larvae of the holoblastic Anamnia such as the frog quickly learn to fend for themselves and therefore have a very small store of nourishment easily housed within the ventral wall of the gut.

In the meroblastic elasmobranchs there is a considerable store of nourishment to tide the developing young over the much longer period preceding the acquisition of independence. The larger yolk mass is for a time extra-embryonic. It is encircled by the endodermal cells, forming a primitive gut or enteron, and later enclosed by the extra-embryonic blastoderm. The yolk sac then becomes connected to the embryo by a constricted narrow part—the yolk duct; this in turn has its covering of mesoderm and ectoderm, and constitutes the somatic stalk. In the final stages the yolk sac is drawn into the body and the somatic stalk is incorporated in the body wall.

The umbilical cord of the Amniota constitutes the pathway for the transit of substances to and from the embryo. In birds and reptiles the extra-embryonic blastoderm (false amnion and endoderm) grows round and encloses the large yolk mass carrying with it an extension of the coelom between the somatopleure and splanchnopleure. In Amniota the allantois issues from the hindgut on the caudal and ventral aspect of the embryo. The true amnion, attached round the embryonic "rim", forms the boundaries of the somatic stalk which thus includes the yolk stalk, a portion of the exocoelom and more caudally the allantoic stalk.

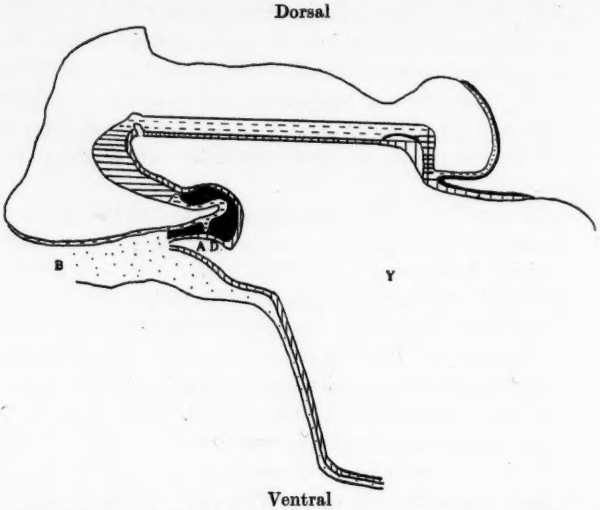
The somatic stalk or umbilical cord of placental mammals is essentially of a similar nature to that described above, although the now increased functional importance of the allantoic element overshadows that of the yolk stalk.

This work consists of a study of the umbilical cord in the series of human embryos already utilized in previous publications (Wyburn, 1937–8), with the

addition of an embryo of 8·5 mm., one of 42 mm., and one of 60 mm. Sections were also made of rabbit embryos in which the gut had returned into the abdominal cavity, but as these show no features of peculiar interest they are not described in detail.

The method of graphic reconstruction is adopted where possible, as it affords a ready appreciation of the relations in any given embryo, and obviates much tedious description of individual sections.

From the observations of the individual embryos one suggests the method of formation of the umbilical cord, completed in human embryos somewhere between the 5 and 7 mm. stage, of the formation and expansion of the umbilical cord coelom which attains its maximum size between the 30 and 40 mm.



Text-fig. 1. Embryo 1·4 mm. (McIntyre I). Reconstruction of a median sagittal section. ×50. Description in text. Horizontal lines = primitive streak. Interrupted horizontal lines = ectoderm. Vertical lines = endoderm, head process and prochordal area. Black = mesoderm. *Y* = yolk sac. *A.D.* = allantoic diverticulum. *B* = body stalk.

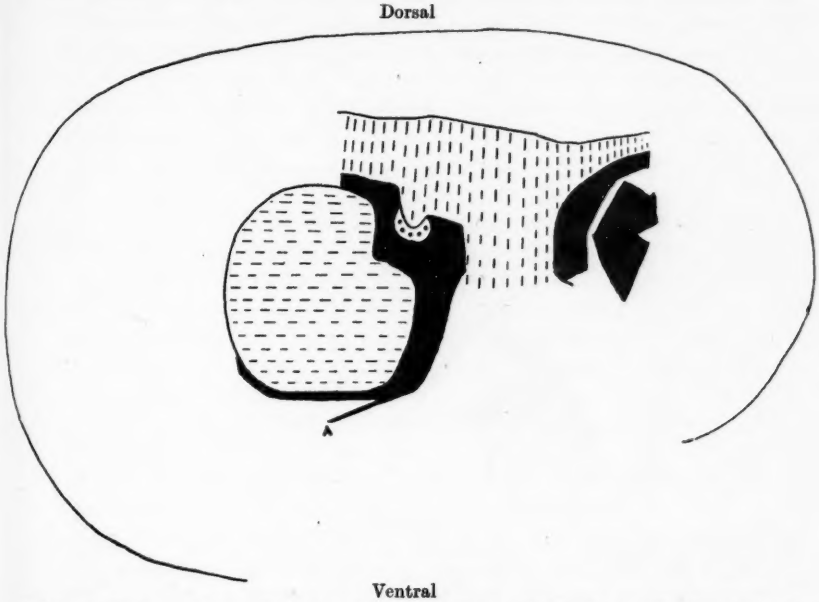
stage, and of the final obliteration of the cord coelom and closure of the umbilical ring subsequent to the return of the gut into the abdominal cavity. No great attention has been paid to the relations and fate of the contents of the umbilical cord such as the yolk duct and vitelline vessels, as these have been recounted with much thoroughness by Politzer & Sternberg in their comprehensive paper "Über die Entwicklung der ventralen Korperwand und des Nabelstranges beim Menschen" to which frequent reference is made throughout.

DESCRIPTION OF EMBRYOS

Embryo McIntyre I (1·4 mm.). This embryo has already commenced to fold off from the yolk sac, and there is a small foregut and larger hindgut diverticulum. The ventral aspect consists mainly of the yolk sac which shows a slight

lateral furrowing indicating the site of later constriction. Cranially the anterior mesodermal field forms the anterior and lateral boundaries of the pericardial plate where the mesoderm exhibits cleavage. Elsewhere there is as yet no intraembryonic coelom.

With the formation of the tail fold the embryonic attachment of the body stalk comes to lie on the ventral aspect. This region has already been described (1937), and it was suggested that the stalk mesoderm was not only derived from primitive chorionic mesenchyme, but received accessions from the caudal margin of the embryonic disc.



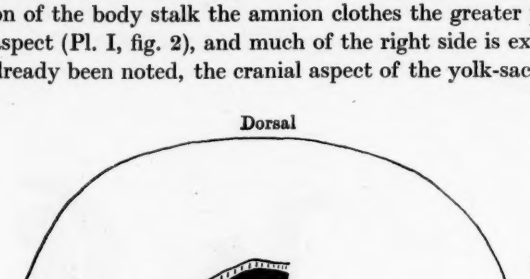
Text-fig. 2. Embryo 2·6 mm. Reconstruction of a median sagittal section. × 50. Description in text. The yolk sac is not shown. Interrupted horizontal lines = pericardial cavity. Interrupted vertical lines = gut. Large dots = liver. Black = mesoderm. *A* = amnion.

Embryo McIntyre I differs from the four-somite embryo of Sternberg in the absence of any extensive connexion of amnion and chorion, i.e. a "primary amnio-chorionic field". There is no amnio-embryonic stalk as described by Florian in presomite embryos. In this respect it can be compared to Embryo Bi. XI (Florian) of ten somites to which it has also some external resemblance.

Embryo 2·6 mm. Following the pronounced involution of the embryonic body the ectodermal surface of the pericardium forms the cranial part of the ventral aspect. There is still a broad yolk-sac connexion which, however, in comparison to the 1·4 mm. embryo has a marked "waisting" at the junction with the midgut.

Between the attachment of the amnion and the cranial aspect of the yolk-sac connexion is a broad band of mesoderm, the anterior mesodermal field, which is continuous dorsally with the splanchnopleure on the floor of the gut. A prolongation of the coelom—the posterior coelomic portal—separates the body stalk from the mesoderm on the caudal side of the yolk-sac connexion. As the tail fold develops this coelomic portal is taken still further into the embryonic body to form the pelvic cavity. The most caudal structure in the somatic stalk is the allantoic or body stalk. This has an oblique attachment, being directed to the left while the yolk sac is to the right. In consequence of this deviation of the body stalk the amnion clothes the greater portion of its left lateral aspect (Pl. I, fig. 2), and much of the right side is extra-amniotic.

As has already been noted, the cranial aspect of the yolk-sac connexion is



Text-fig. 3. Embryo 4·5 mm. Reconstruction of a median sagittal section. × 25. Description in text. Yolk sac is not shown. Interrupted horizontal lines = pericardium. Interrupted vertical lines = gut. Black = mesoderm. Large dots = liver. *C* = coelome. *A* = amnion. *B* = body stalk.

lying against the anterior mesodermal field. There is now a body cavity which has a relatively extensive communication with the extra-embryonic coelom on each side of the yolk-sac connexion, i.e. where the ventral wall remains "open". Each intercoelomic communication is bounded medially by the yolk-sac connexion and laterally by the body wall with the amnial reflexion. At the rim or point of amnial reflexion are the umbilical veins, the left larger than the right. Here the somatopleure of the lateral body wall becomes continuous with the mesoderm of the amnion. There is a marked increase of mesoderm at the site of amnial reflexion (Pl. I, fig. 1).

Embryo 4·5 mm. The further folding of the head and tail ends is responsible for the relative decrease in the extent of the "open" ventral aspect and the increased dorsi-ventral convexity for the greater depth of intra-embryonic

coelom. Moreover, a part of the extra-embryonic coelom has been taken into the body anteriorly as well as posteriorly, and a coelomic prolongation now separates the proximal end of the yolk duct from the anterior mesodermal field and septum transversum (Pl. I, fig. 4). The connexion of the intestine to the yolk sac has become narrowed to a tube-like communication which at this stage could be called the vitelline duct.

The open ventral aspect is limited cranially by the amnion reflected on to the caudal wall of the completely rotated pericardium, and caudally by the allantoic stalk[1] which in this embryo has a more or less median position.

The lateral boundaries are the somatopleure of the body wall and more ventrally the thickened mesoderm on the inner aspect of the amnion. This thickening of the amnial mesoderm is much more apparent than in the 2·6 mm. embryo. In its forward growth it carries with it the reflexion of the amnion on each side and forms lateral tissue plates[2] which extend from the "stalk" to the septum transversum (Pl. I, fig. 3). There is therefore in this embryo a part of the extra-embryonic coelom enclosed between the stalk, the cranial reflexion of the amnion and the lateral tissue plates, i.e. there is a short umbilical cord.

The earlier site of lateral amnial reflexion is indicated by the position of the umbilical veins, each of which runs in a ridge of tissue projecting from the internal aspect of the somatopleure. This is also the junction of the lateral body wall with the lateral tissue plates and of the exocoelom with the body cavity. The lateral tissue plates consist externally of amnial ectoderm, internally of endothelium from the amnial mesoderm and a thick middle coat of proliferated embryonic connective tissue. This young connective tissue may result from migration of somatopleure cells, but more probably is a derivative of the amnial mesoderm. The lateral tissue plates are deeper caudally and "taper" towards the septum transversum, so that in the wax reconstruction they have the appearance of cranial extensions or pillars of the "stalk".

The smooth mesothelium of the cranial aspect of the stalk forms the caudal lining of the enclosed exocoelom. An umbilical loop of intestine is not yet formed.

Embryo 7 mm. This is the youngest embryo of the series to possess a formed umbilical cord. Coincident with this is the first appearance of a short supra-umbilical abdominal wall, and thus the cranial attachment of the cord is a little distance caudal to the pericardium.

Comparison of Text-figs. 3 and 4 gives some indication of the changes by which the "open" ventral wall of the embryo is now the site of attachment of the umbilical cord.

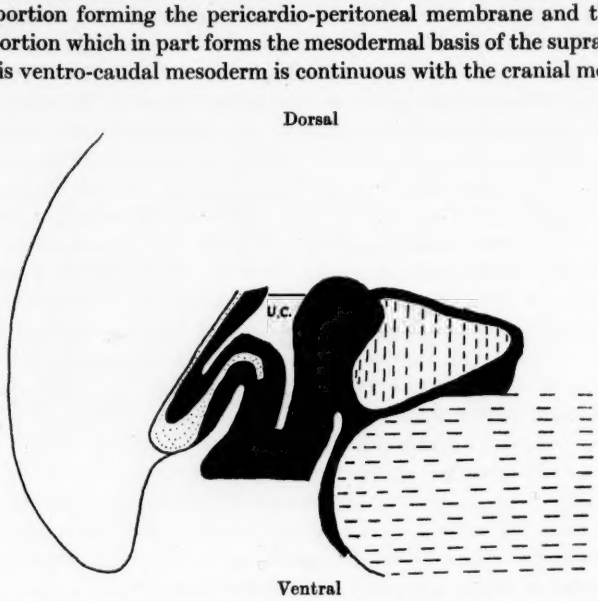
The lateral tissue plates whose formation had commenced in the 4·5 mm. embryo undergo further growth by proliferation of their connective tissue, and in their ventral extension carry with them the amnial reflexion. There is also

[1] From now on the allantoic stalk will be referred to as "stalk".

[2] The term "lateral tissue plate" shall be used to denote the amnion with its thickened mesoderm ventral to the umbilical veins.

proliferation of the mesoderm at the cranial reflexion of the amnion. Therefore the amnion at its embryonic attachment now encloses a circular band of embryonic connective tissue made up of the "stalk" caudally, the lateral tissue plates on each side, and cranially the proliferated amnial mesoderm—the umbilical cord.

The anterior mesodermal field, which in the 4·5 mm. embryo is caudal and dorsal to the pericardium, is here divided by the developing liver into the cranial portion forming the pericardio-peritoneal membrane and the ventro-caudal portion which in part forms the mesodermal basis of the supra-umbilical wall. This ventro-caudal mesoderm is continuous with the cranial mesoderm of



Text-fig. 4. Embryo 7 mm. Reconstruction of a median sagittal section. × 25. Description in text. Interrupted horizontal lines = pericardium. Interrupted vertical lines = liver. Small dots = gut and allantoic diverticulum. *U.C.* = umbilical cord coelom. Black line indicates the plane of the umbilical veins.

the umbilical cord, the two together forming an *intercoelomic septum* which separates the umbilical cord coelom from the liver ventrally and from the body cavity more dorsally (Pl. II, fig. 5).

The umbilical veins run in the dorsal part of the intercoelomic septum to reach the liver (Pl. II, fig. 6).

The completed cord includes a part of the exocoelom—the umbilical cord coelom. In this embryo it consists of a wide proximal portion and a narrow slit-like prolongation (Pl. II, figs. 6, 7). The proximal portion has a caudal extension between the gut and the allantois, and dorsally a wide communication with the intra-embryonic coelom. It is bounded caudally by the "stalk", cranially by the intercoelomic septum, and on each side by the lateral tissue plates.

In a transverse section of the cord some distance distal to its attachment, there are a number of large spaces in the loose embryonic connective tissue. One of these spaces contains blood clot and has a distinct but not an endothelial lining (Pl. II, fig. 7). These large spaces result from confluence of a number of smaller ones. The umbilical cord coelom increases considerably in size in older embryos, very probably by the "taking in" of these spaces and a proliferation and extension of the endothelial lining. Politzer & Sternberg remark on the normal occurrence of spaces in cord tissue and state "über ihre Bedeutung können nur Vermutungen ausgesprochen werden, doch scheint es uns möglich, dass ihre Auftreten, zumindest im proximalen Abschnitte des Nabelstranges, mit der Vergrösserung der Nabelstrangcoelomhöhle in Zusammenhang steht".

The midgut is differentiated and there is a definite loop of intestine in the umbilical cord coelom.

In Text-fig. 4 the position of the umbilical veins is indicated and this also represents the division between the intra- and extra-embryonic coeloms. It will be observed that the gut is lying in the ventral part of the body cavity so that in its growth it almost inevitably protrudes into the umbilical cord coelom.

The cranio-caudal width of the cord at its point of attachment to the embryo is 0·5 mm.[1] The length of the attachment cranial to the umbilical cord coelom is 0·24 mm., which is, in fact, the cranio-caudal measurement of the intercoelomic septum,[2] while the wide umbilical cord coelom measures 0·4 mm.

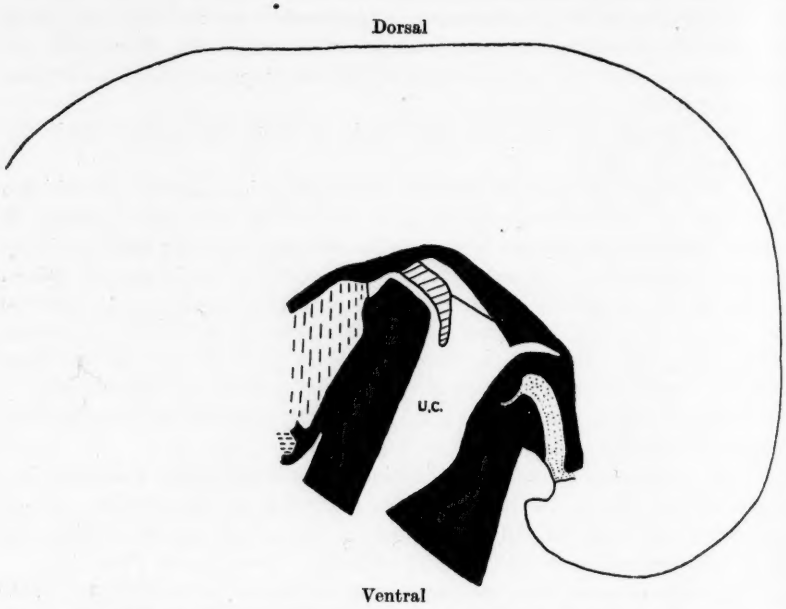
Embryo 8·5 *mm.* This embryo has been recently acquired and therefore was not previously described.

The umbilical cord has an inclination towards the right, and there is a small yolk sac also on the right side. The embryo is cut transversely, and the umbilical cord longitudinally. The cord extends almost to the chorion, but there is a small extra-amniotic stretch of "stalk" distally and to the left.

The ventral aspect has straightened out somewhat compared to that of the 7 mm. embryo. This is due to the forward and caudal extension of the liver and the formation of a longer supra-abdominal wall. The cranial portion of the cord consists of a loose embryonic connective tissue which, dorsal to the cord attachment, fuses with the denser tissue on the ventral aspect of the liver to form the intercoelomic septum (Text-fig. 5). It should be noted that with the stretching of the ventral wall the intercoelomic septum is no longer in the dorsiventral plane but makes an angle of about 45° with it and thus the cord is tending to become "prised out" of the embryonic body. The length of the cord attachment is 1·2 mm., and of its cranial attachment 0·6 mm. This increase (cf. 7 mm.) is in fact the result of the more horizontal position of the intercoelomic septum. The widest part of the umbilical cord coelom is at its communication with the body cavity, the coelomic portal,[3] and measures 0·6 mm.

[1] Will be referred to from now on as "cord attachment".
[2] Will be referred to from now on as "cranial attachment" of cord.
[3] The communication of extra- and intra-embryonic coeloms.

In a longitudinal section of the cranial half of the cord (Pl. II, fig. 8) can be observed the distal prolongation of the umbilical cord coelom containing the vitelline stalk. This part of the coelom is much larger than the corresponding extension in the 7 mm. embryo. On the left the amnion is reflected from the cord while still a short distance from the chorion, and at the point of reflexion the endothelium of the coelom becomes continuous with the mesothelium of the amnion. Proximal to this point the endothelium and amnial ectoderm are



Text-fig. 5. Embryo 8·5 mm. Reconstruction of a median sagittal section. × 25. Description in text. Interrupted horizontal lines = pericardium. Interrupted vertical lines = liver. Oblique lines = blood vessels. Black = mesoderm. Small dots = gut. *U.C.* = umbilical cord coelom. Black line indicates the plane of the umbilical veins.

separated by a thickness of mesenchyme—the proliferated connective tissue of the lateral tissue plates.

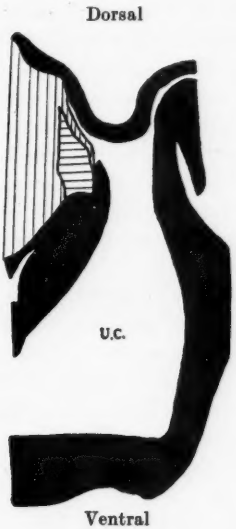
A noticeable feature in this embryo as in the 7 mm. one is the fact that the umbilical cord actually penetrates the embryonic body for some distance, and accordingly there is a relatively shallow intra-embryonic coelom at this level. It is therefore not surprising that the growing intestine should find its way into the extra-embryonic coelom.

The liver still occupies but a small part of the abdominal cavity, and can have little or no influence on the position of the gut at this time.

Embryo 12·5 *mm.* The cord attachment extends over 1·9 mm. and the

cranial attachment is over 1 mm. The increased length of the cranial attachment is simply due to the more horizontal position of the intercoelomic septum which in turn has been brought about by the caudal and ventral extensions of the now rapidly growing liver. As a consequence of both these factors the cord is still further "prised" out of the embryonic body, and the body cavity has a much increased dorsi-ventral depth. Above the cord is the supra-umbilical area, and caudal to its lower attachment is a small infra-umbilical area of abdominal wall.

Text-fig. 6.



Text-fig. 7.

Text-fig. 6. Embryo 12·5 mm. Reconstruction of a median sagittal section. × 13. Description in text. Interrupted horizontal lines = pericardium. Interrupted vertical lines = liver. Small dots = gut. Oblique line = blood vessels. Black = mesoderm. *U.C.* = umbilical cord coelom.

Text-fig. 7. Embryo 14 mm. Reconstruction of a median sagittal section. × 13. Description in text. Vertical lines = liver. Horizontal lines = blood vessel. Black = mesoderm. *U.C.* = umbilical cord coelom.

The coelomic portal has a cranio-caudal width of 0·5 mm., but a short distance distal to this the umbilical cord coelom widens out to 1·1 mm. There is thus in this embryo a bottle-neck communication between the intra- and extra-embryonic coeloms; a characteristic feature in all the older specimens.

A section at the level of the cranial attachment of the cord (Pl. III, fig. 9) shows the myotomic downgrowths fusing in the mid-line ventrally to form a denser connective tissue on the superficial aspect of the umbilical vein. At the level of the coelomic portal the downgrowths are not so definite and do not reach the sides of the cord. The stalk tissue with the vessels is on the right side of the cord.

The significance of the zone of mesodermal condensation on the caudal aspect of the cord has already been discussed (1937).

Embryo 14 *mm.* In this embryo the umbilical cord in the first part of its course turns towards the head and the umbilical vessels occupy the left aspect. The extent of cord attachment measures 1·6 mm.—an absolute and relative decrease from the corresponding figure in the preceding embryo. The cranial attachment is 0·8 mm., while the intercoelomic septum is now lying at an angle of about 45° to the horizontal.

A narrow coelomic portal (cranio-caudal width 0·6 mm.) is the entrance into a large umbilical cord coelom which but a short distance from the cord attachment has expanded to a cranio-caudal breadth of 2 mm., and is responsible for the increased circumference of this part of the umbilical cord.

Although not so well marked as in the 12·5 mm. embryo, the myotomic downgrowths can be discerned in the upper half of the abdominal wall and traced into a mesodermal condensation on the anterior aspect of the umbilical vein (Pl. III, fig. 10). In the mid-abdominal region, however, i.e. at the level of the coelomic portal, the downgrowths fade away before reaching the ventral aspect.

Embryo 16·1 *mm.* The area of cord attachment is 1·3 mm., the cranial attachment 0·5 mm., and the coelomic portal 0·7 mm.

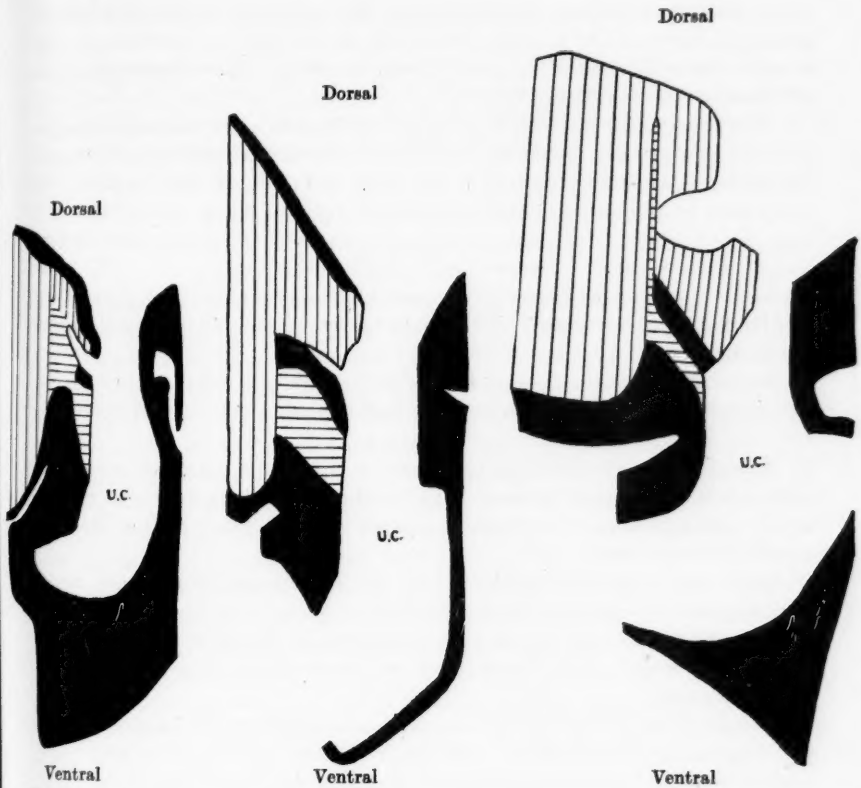
Above the cord the mid-ventral wall is a narrow band of loose embryonic connective tissue. At the level of the cranial attachment of the cord the mesoderm ventral to the liver and the immediate cord tissue (Pl. III, fig. 11) is of a more compact texture, and this denser connective tissue can also be observed extending some little way into the pillars of the cord flanking the coelomic portal.

Embryo 23 *mm.* The extent of umbilical cord attachment is 2 mm., the cranial attachment is 0·7 mm., and the cranio-caudal width of the coelomic portal is 1 mm. In this embryo the intercoelomic septum is in the horizontal plane, and the liver in the mid-ventral line encroaches on the cranial half of the coelomic portal. A little distal to the embryo the cord coelom has a cranio-caudal measurement of 1·6 mm.

In the upper part of the abdominal wall the mesoderm of the mid-ventral line is of a loose texture, but at the level of the cord attachment this is replaced by a compact tissue which is continuous on the one hand with the myotomic downgrowth (Pl. III, fig. 12) and on the other extends into the proximal part of the intercoelomic septum; and more caudally into the pillars of the cord on each side of the coelomic portal. Along with the condensation of mesoderm on the caudal aspect of the cord there is thus a ring of compact tissue at the cord attachment which forms the circumference of the coelomic portal.

Embryo 40 *mm.* The extent of cord attachment extends over 2 mm. of the ventral body wall. There is a very small cranial attachment of 0·4 mm. and a coelomic portal of 1·3 mm. opening into a wide umbilical cord coelom which, however, on account of the obliquity of the section through the cord, could not

be measured. From the embryo the umbilical cord takes a cranial direction, and its coelom, with the endothelial lining, can be followed as a narrow prolongation into the more distal sections.



Text-fig. 8. Text-fig. 9. Text-fig. 10.

Text-fig. 8. Embryo 16·1 mm. Reconstruction of a median sagittal section. × 13. Description in text. Vertical lines = liver. Horizontal lines = blood vessels. Black = mesoderm. *U.C.* = umbilical cord coelom.

Text-fig. 9. Embryo 23 mm. Reconstruction of a median sagittal section. × 13. Description in text. Vertical lines = liver. Horizontal lines = blood vessels. Black = mesoderm. *U.C.* = umbilical cord coelom.

Text-fig. 10. Embryo 40 mm. Reconstruction of a median sagittal section. × 10. Description in text. Vertical lines = liver. Horizontal lines = blood vessels. Black = mesoderm. *U.C.* = umbilical cord coelom.

The liver occupies an even greater proportion of the abdominal cavity than in the 23 mm. embryo, and in the mid-line encroaches not only ventrally but

also dorsally on the cranial half of the coelomic portal. At this level the intra-abdominal part of the intestine is lodged in a groove between the above ventral and dorsal portions of the liver. There is a broad linea alba in the supra-umbilical abdominal wall formed by a fusion of the sheaths of the recti muscles which provides a fibrous condensation on the superficial aspect of the intra-abdominal part of the umbilical vein. At the attachment of the cord the muscle sheaths fuse with the umbilical ring of compact tissue surrounding the coelomic portal (Pl. IV, fig. 13).

Embryo 42 *mm.* This embryo is included in the series as it demonstrates the presence of an empty umbilical cord coelom after the withdrawal of the gut. The cord coelom is much reduced in size from that in the 40 mm. embryo, and is occupied by a quantity of blood clot (Pl. IV, fig. 14). In the more distal part there is an absence of the endothelial lining; the wall is irregular and formed simply of the connective tissue of the cord (Pl. IV, fig. 15).

In the proximal cord coelom the endothelial cells are being separated off (Pl. IV, fig. 16) preparatory to the ultimate degeneration and disappearance of the endothelium.

The anterior sheath of the recti muscles is continuous on all sides with the compact tissue encircling the now much narrowed coelomic portal (Pl. IV, fig. 14).

Embryo 60 *mm.* A description of the relevant sections will be given as these include only the anterior abdominal wall in the region of the cord attachment, and accurate graphic reconstruction was not possible. The length of the cord attachment is 3·2 mm.

In the supra-umbilical region (Pl. V, fig. 17) the now well-formed broad linea alba lies superficial to the umbilical vein whose coats are differentiated. Posteriorly between the vein and the peritoneum is a layer of fibrous tissue—the umbilical fascia—the variations and significance of which have been worked out by Gorelow.

The character of the tissue of the umbilical cord shows considerable change from that in the 40 and 42 mm. embryos (Pl. V, fig. 18). There has been much proliferation of embryonic connective tissue which approximates to adult fibrous tissue. This proliferation is more apparent at the attachment of the cord in the vicinity of the blood vessels. The coats of the vessels themselves have increased in thickness, and the allantoic arteries have a layer of visceral muscle. In the distal sections of the cord there is a less dense and more open appearance. On each side the sheaths of the recti muscles are continued into the broad band of compact fibrous tissue which forms the cranial attachment of the cord, lies on the superficial aspect of the umbilical vein, and can in turn be followed into the supra-umbilical linea alba (Pl. V, fig. 18).

At the proximal end of the cord the umbilical vein takes the left and cranial side, while the allantoic structures are on the caudal and right aspects.

Pl. V, fig. 19, is a photograph of a section about midway through the cord attachment. The muscle sheaths run into the cord tissue on each side. In this

embryo there is no umbilical cord coelom and the body cavity is shut off from the cord by the peritoneum.

At the caudal end of the cord attachment (Pl. V, fig. 20) there is a band of compact tissue superficial to the allantoic arteries and allantois. This merges on each side with the sheath of the rectus muscle and caudally is continued into the infra-umbilical division of the linea alba.

In this embryo the encircling band of compact tissue at the cord attachment is a particularly noticeable feature, and by its proliferation is responsible not only for the closure of the umbilical ring, but also a reinforcement of the immediate abdominal parietes.

DISCUSSION AND CONCLUSIONS

Formation of the umbilical cord

The umbilical cord is formed essentially by the closing in of the somatic stalk. In young embryos the somatic stalk is bounded by the attachment of the amnion round the broad ventral aspect. The approximation of the amnial reflexions is brought about by the ever increasing involution of the embryonic body. Following rotation of the pericardium the cranial reflexion of the amnion moves from the ventral to the caudal pericardial wall, and finally at the 7 mm. stage the amnion is reflected on to the newly formed supra-umbilical portion of the abdominal wall. The allantoic stalk covered posteriorly and laterally with amnion is rotated ventrally and while part of the stalk is thereby taken into the body of the embryo the relative distance between the caudal and cranial amnial reflexions, i.e. the breadth of somatic stalk, is decreased. In addition there is a relative narrowing in the transverse axis as a result of the formation of the vitelline duct coupled with the great dorsi-ventral convexity of the embryonic body.

The lateral tissue plates make their first appearance in the 4·5 mm. embryo. They arise as a proliferation of the embryonic connective tissue between the ectoderm of the amnion and its mesothelial or endothelial lining, and in their forward growth they carry with them the lateral amnial reflexions which will now lie some distance ventral to the umbilical veins and thus ventral to the embryonic rim. They connect the allantoic stalk to the septum transversum and represent the formation of the umbilical cord.

By a proliferation of the mesoderm the tissue plates continue to grow in length and thickness. There is also an increase of the corresponding mesoderm at the cranial reflexion of the amnion which might be said to form a third or anterior tissue plate. At a later stage—the 7 mm. embryo—a portion of the exocoelom has been enclosed by these thick endothelial lined tissue plates which encircle it laterally and in front. The caudal boundary is the cranial surface of the body stalk.

This is the umbilical cord and it finally stretches from the embryo to the chorion.

Umbilical cord coelom

In any given embryo the level at which the umbilical vein enters the body wall indicates the boundary between intra- and extra-embryonic coeloms.

In the 2·6 mm. embryo there is, on the ventral aspect, a wide communication between the intra- and extra-embryonic coeloms. Posteriorly the coelom extends as a caudal recess some distance into the body of the embryo. This caudal recess is extra-embryonic coelom which has "rotated" into the embryonic body as the tail fold forms. Anteriorly the coelomic portal has the opening of the coelomic tubes on each side of the mid-line. There is as yet no median prolongation of the coelom and the cranial wall of the yolk-sac connexion lies against the septum transversum (Text-fig. 2). With the further involution of the embryo (e.g. embryo 4·5 mm.) the cranial wall of the now constricted yolk-sac connexion becomes separated from the septum transversum by an extension of the coelom, and a short length of gut intervenes between them.

The intra-embryonic coelom is therefore to some extent increased by the inclusion of part of the exocoelom.

The umbilical cord coelom in the first instance consists of a narrow slit-like distal part and a wide proximal part which receives the opening of the caudal recess and communicates with the body cavity. It enlarges, *pari passu*, with the growth of the umbilical loop of intestine until it attains a maximum size in embryos between 30 and 40 mm. In these older specimens the umbilical cord coelom occupies a proportionately greater extent of the cranio-caudal diameter of the cord, and therefore some of this increase in size is at the expense of cord tissue. The opinion has already been expressed that there is a confluence of spaces normally found in the cord mesenchyme, that these open into a cord coelom, and the endothelium extends into and forms a lining for them.

The coelomic portal is in the earlier stages the widest part of the cord coelom. It, however, soon becomes relatively narrow and constitutes a "bottle-neck" intercoelomic passage.

Eventration of the gut

In the 4·5 mm. embryo there is a short length of gut between the cranial margin of the yolk-sac connexion and the septum transversum. This is the first indication of a mid-gut loop and it is intra-embryonic. The proximal umbilical cord coelom, in the 7 mm. embryo, contains the umbilical loop of intestine, and there it remains and develops till about the 42 mm. stage.

Why should a portion of intestine grow for a period outside the body of the embryo? To state that it does so because of lack of space within the abdominal cavity leaves still unexplained this apparent temporary architectural miscalculation. There seems no adequate reason why the intestine should not grow as well if not better inside an abdominal cavity of the requisite size. "Es sieht daher tatsächlich hier so aus, als ob die Natur unnötigerweise eine konstante embryonale Missbildung hervorriefe, die sie nachher die Mühe haben müsse, wieder zu reparieren" (Broman).

There are two aspects of the problem of what has been described as the physiological hernia.

First, the method of production—in other words, how does the hernia occur? The various theories and suggestions appertaining thereto are the result of the study of the relevant factors in the developing human embryo. Secondly, why does the hernia occur? Observation of human ontogeny alone would leave this question still unsolved, and the final answer is only found in terms of comparative embryology.

The method of production

The most apparent factor in the production of the hernia is the connexion of the intestine with the yolk sac. In the early stages the broad yolk-sac connexion maintains the gut in the ventral portion of the relatively shallow body cavity, so that when the mid-gut develops by constriction of the yolk sac it very quickly comes to lie in the proximal part of the umbilical cord coelom which at that time has a wide communication with the body cavity (Text-fig. 4). As the embryo becomes folded off and the body cavity enlarges there would be a tendency for the umbilical loop to re-enter the intra-embryonic coelom, but it is maintained in or actually drawn farther into the exocoelom by the yolk stalk. The vitelline duct itself becomes obliterated, according to Politzer & Sternberg, between the 7 and 9 mm. stage, and soon after severs its connexion with the intestine. There, however, still remains attached to the ileal mesentery the mesodermal yolk stalk containing the vitelline artery which does not disappear till about the 15 mm. stage (Broman), and this is an important factor in the maintenance of the extra-embryonic position of the gut. In the present series of embryos there is no trace of a yolk stalk in the 7 mm. embryo, but it is present very definitely in the older 8·5 mm. specimen. In a rabbit embryo in which the gut has returned to the abdominal cavity, there is still present a mesodermal stalk connecting the mesentery to the umbilical cord. Broman states that in the cat and the seal the yolk stalk persists for a much longer period than in the human.

It has been suggested (Mall) that the rapidly developing liver "squeezes" the gut into the exocoelom, but the umbilical loop of intestine can be observed in its extra-embryonic position at a stage, e.g. 7 mm., when the liver occupies only a small proportion of the abdominal cavity. The later great extension of liver tissue combined with the narrow intercoelomic communication will certainly help to maintain the extra-embryonic position of the gut, but hepatic growth is not responsible for its initial production.

Why should the hernia occur?

The primitive gut or enteron is formed by an extension of the endoderm round the yolk mass. In amphibians, such as the frog, the enteron with enclosed yolk is within the body of the quickly formed larva.

Where the yolk furnishes nourishment over a more prolonged period, as in elasmobranchs, reptiles and birds, it would obviously be impracticable to harbour this inert mass within a growing embryo, and so the original plan is modified—the yolk sac remains for a time extra-embryonic and lies within an extra-embryonic extension of the coelom. The large and heavy yolk sac acts as a drag on the intra-embryonic intestine to which it is attached, and this portion will be brought without the embryo to form the umbilical loop in the exocoelom.

In reptiles and birds the yolk sac is taken into the body of the embryo at a late stage—the umbilical cord coelom is incorporated in the body cavity and its somatopleure with the body wall.

In placental mammals the role of the yolk sac is of comparatively minor significance and confined to the early stages, but it still plays a part, however transient, in the physiology of the embryo, and its extra-embryonic modification serves as a basis for still more elaborate nutritional preparations. As a concomitant of the extra-embryonic position of the yolk sac there is a portion of the definitive intestine in the umbilical cord coelom. The part played by the yolk sac is soon over; its further development or inclusion within the embryo would serve no useful purpose. It therefore atrophies and severs its connexion with the intestine which it leaves within the exocoelom.

The physiological hernia does not therefore occur *a priori* but rather is incidental to the extra-embronic position of the yolk sac. It is a by-product of a plan which utilizes part of the primary enteron as a storehouse for nutriment. In mammals much of this scheme is still further modified, but, as the presence of the hernia indicates, it is not altogether discarded.

Later changes in the form of the umbilical cord

In the stages immediately succeeding the formation of the umbilical cord there is an increase absolute and relative in the cranio-caudal length of its embryonic attachment, for whereas at 7 mm. this area is one-fourteenth of the total embryonic length it accounts for one-seventh of the total length at 8·5 and 12·5 mm. After this the rate of growth lags behind that of the embryo as a whole, and at 42 and 60 mm. the proportion is only one to twenty. The umbilicus can therefore be regarded as a relatively fixed area.

At the level of its proximal attachment the umbilical cord consists of the embryonic connective tissue of the intercoelomic septum cranially and the stalk mesoderm caudally, which, together with the lateral amnial plates, form the respective boundaries of the umbilical cord coelom.

The relative proportion of cord structure formed by intercoelomic septum and coelomic portal undergoes a change as development proceeds. The former, which at an early stage forms more than half, is, in the older embryos, reduced to less than half of the sagittal diameter of the cord at its proximal attachment, while the latter is increased accordingly. This increase, however, is by no means commensurate with the enlargement of the more distal cord coelom, and the result is a narrow bottle-necked intercoelomic communication.

The intercoelomic septum is situated in the first instance with its long axis dorsi-ventrally, and the cord is "sunk" for quite a considerable distance into the embryonic body. With the ventral and caudal extension of the liver the intercoelomic septum is rotated through 90° so that its long axis is now in the cranio-caudal plane and the cord is "eased out" of the body of the embryo.

Dense embryonic connective tissue encircles the attachment of the cord. It forms an umbilical ring of mesodermal condensation surrounding the coelomic portal, and is present in the 16·1 mm. embryo, but more emphatically so in the 23 mm. embryo. The compact tissue first appears in the stalk mesoderm, situated superficial to the allantoic vessels. Cranially it lies ventral to the umbilical vein and on each side extends into the tissue of the lateral pillars of the cord bounding the coelom. When the myotomic downgrowths reach the ventral aspect their anterior portions, i.e. the sheaths of the recti muscles, become continuous with the tissue of the umbilical ring.

Ventralization of the gut

It is generally agreed that there is a comparatively rapid passage of the umbilical loop of intestine into the abdominal cavity in embryos of from 40 to 42 mm. I have been unable to find described any intermediate stage with a partial reduction of the gut, but nevertheless it is obvious, as stated by Frazer, "that there is no question of the entrance of the contents of the sac en masse into the abdomen", but that the coils of gut "slip back in a continuous movement".

In the chick the large yolk sac together with the sac containing the intestine is taken into the body of the embryo about the 19th day. The distal wall of the yolk sac, according to Lille, is fused with the allantois. This part of the allantois has a muscular wall, and by its contraction initiates the propulsion of the yolk sac towards the embryo. The movement is completed by the pressure of the inner wall of the amnion which possesses an interlacing system of muscle fibres. The umbilicus is closed by the act of inclusion (except for a small central aperture) as the inner wall of the amnion is attached on the one hand to the body wall and on the other to the distal pole of the yolk sac.

There are no muscular fibres in the mammalian amnion nor any other means of exerting pressure on the umbilical sac from without. Therefore it must be assumed that there is an intra-embryonic influence sufficiently effective to overcome the resistance of the narrow inlet. This could be (A) of the nature of a direct pull, or (B) a suction force by the establishment of a negative abdominal pressure (i.e. negative relative to that in the exocoelom).

Under A have been cited (1) a rotation, and increase in size, of the intra-abdominal loop of intestine (Mall); (2) shortening of the vitelline vessels (Rathke); (3) a dorsal pull exerted on the gut by a strong caudal growth of the liver (Broman).

A negative intra-abdominal pressure would be caused by the expansion of the abdominal cavity and diminished growth rate of liver (Frazer). Certainly

coincident with the entrance of the gut there is a decrease in the size of the liver, but this might be the effect rather than the cause, as the pressure of additional gut would cancel the negative pressure and allow the liver to "contract".

The study of this series of embryos has failed to reveal any additional factor which would further aid in a solution of the problem.

As far as the question lends itself to elucidation in terms of physical forces the evidence favours the creation of a negative abdominal pressure and a "suction" influence rather than a direct pull.

To be reckoned along with any mechanical explanation, however plausible, of embryological phenomena, is the biological urge towards the completion of a specific form which finds its modern expression in the terms "organiser", "growth centre", etc., and which so far has defied all effort to place it within any known hierarchy of physical laws.

Obliteration of the umbilical cord coelom and closure of the umbilical ring

The passage of the umbilical loop of gut into the abdominal cavity leaves a still open coelom in the umbilical cord, as has been observed in the 42 mm. embryo. Apart from this direct evidence of the presence of the empty exocoelom, any question of its incorporation in the embryonic body would be negatived by the following observations: (*a*) the extent of the cord coelom in the older embryos (23 and 40 mm.), (*b*) its prolongation into the distal cord, and (*c*) the bottle-necked coelomic portal.

The first step towards obliteration is probably a certain amount of collapse of the wide proximal cord coelom, then a degeneration of the endothelial lining (Pl. IV, fig. 16) would permit of the filling up of the space by the proliferation of contiguous fibrous tissue. Such an increase of connective tissue is strikingly apparent in the 60 mm. embryo, where there is not only a greater density of tissue, but it is of a more mature type. It is particularly concentrated in the region of the proximal attachment of the cord, and could be regarded as the result of increased activity of the ring of fibrous tissue already described. This proliferating zone is responsible for the closure of the umbilical ring and forms a collar encircling the base of the cord and extending into the abdominal wall. Above and below it plays a part in the formation of the supra- and infra-umbilical portions of the linea alba, and is continued into and reinforces the rectus sheath on each side.

It is perhaps of some significance that in the human and probably other placental mammals the umbilical cord as in some lower vertebrates makes its contribution to the body wall, and that this contribution traced to its ultimate source is a derivative of the primary mesoderm.

Congenital herniae due to maldevelopment of the umbilical region

Recorded in the literature are many and varied forms of congenital deficiencies of the anterior abdominal wall with a correspondingly formidable array of names and a somewhat confusing choice of classification.

Ectopia vesicae and absence of an anterior abdominal wall (eventration) have already been discussed (1937, 1938), and here remarks will be confined to herniation which results from maldevelopment of the umbilical cord and neighbouring parietes.

A. *Arrested formation of the umbilical cord.*

In the Hunterian Pathological Collection of Glasgow University is a specimen (no. 50–113) of a 6 months foetus labelled "Hernia into the umbilical cord". The greater portion of the abdominal wall is occupied by a large thin-walled sac about the size of a hen's egg which contains most of the abdominal viscera. A stalk or cord arises from the apex of the sac. There is no neck to the sac, but a wide circular aperture opens into the body cavity proper: this is the Exomphalos of Fraser's classification. In the specimen there is a supra- and infra-umbilical portion of the abdominal wall and some attempt at closure of the somatic stalk to form an umbilical cord (cf. cases of eventration), but the band of mesodermal condensation normally present at the embryonic attachment of the cord is absent, and in consequence there is no proper umbilical ring and the coelomic portal has expanded *pari passu* with the exocoelom.

B. *Persistence of the physiological hernia.*

Specimen no. 50–112 of the Hunterian Collection is also labelled "Hernia into the umbilical cord", but is an entirely different condition from the preceding example. The large sac, which has been opened, contains the major portion of the gut including the caecum and appendix, and communicates with the intra-embryonic coelom by a narrow constricted neck. The intestinal wall is firmly adherent to the upper rim of this umbilical ring. Thunig thinks that adhesion of the intestine to a patent umbilical ring is a primary aetiological factor in persistent physiological hernia. It is equally possible that a premature narrowing of the coelomic portal by a precocious proliferation of the fibrous tissue of the umbilical ring might defeat attempts at reduction. With the growth of the contained gut and a further relative constriction of the neck a condition akin to incarceration would exist, and secondary inflammatory adhesions develop.

It should be noted that as the caecum and appendix are normally the last parts of the gut to enter the abdominal cavity they will perforce be included in the hernial sac in all cases of the above condition.

C. *Non-closure of the umbilical ring.*

If the umbilical ring remains patent there may be a persistent umbilical cord coelom, or a new peritoneal sac may be formed. In either case the entrance into the sac of a loop of bowel will result in a hernia into the umbilical cord which, of course, occurs after the reduction of the physiological hernia. Such a hernia may be symmetrical or asymmetrical, depending on the position of the allantoic stalk in the cord. Clinically this is the commonest type of

umbilical hernia and is the "simple umbilical hernia" of Schwalbe, the "small umbilical hernia" of Politzer & Sternberg, the postnatal umbilical hernia of Fraser, and includes "congenital hernia" into the cord and infantile hernia (R. Maingot).

It has been shown that the closure of the umbilical ring is effected by a proliferation of the encircling band of compact mesoderm. Partial failure or absence of this proliferation would leave a patent umbilical ring—a potential hernial site.

Defects of the parietes in the umbilical region

From observations on the older embryos of the series, one concluded that there is a contribution of mesoderm from the tissue of the umbilical cord to the abdominal parietes.

The linea alba immediately above and below the cord attachment and the sheath of the rectus muscle on each side are formed by a fusion of the anterior portion of the myotomic downgrowths with connective tissue from the compact condensation round the base of the umbilical cord. It is suggested by Schwalbe that partial deficiencies of the abdominal wall are due to a mesodermal failure of one or two particular somites related to a scoliotic deformity of the vertebral column. Difficult to reconcile with this view is the fact, also stated by Schwalbe, that the abdominal muscles are normal and the recti form the lateral boundaries of the parietal deficiency.

A defective proliferation or insufficiency at one or other point of the umbilical ring of compact mesoderm would result in a weakness of the corresponding part of the umbilical region of the abdominal wall. This might affect the linea alba above or below the umbilicus, or the immediate lateral parietes—all of them sites of potential hernia. Such a condition has been variously designated as amniotic umbilicus (Tennant), amniotic linea alba (Thunig), or, where a hernia has developed, amniotic hernia (Freshman).

An aggravation of the deficiency in the subumbilical region is exemplified in ectopia vesicae, while its counterpart above would cause a condition approaching partial eventration. Perhaps the most common result of such a parietal defect is a small asymmetrical hernia above or to one or other side of an apparently normal umbilical cord.

In each and all of the above malformations leading to herniae in this region of the abdominal wall, one is of the opinion that the initial error or fault can be traced to that mesoderm which by its proliferation forms the amnial tissue plates of the umbilical cord, closes the umbilical ring, and contributes to the umbilical part of the abdominal parietes. The earlier the flaw the more serious the consequences, as in severe forms of exomphalos, while its later occurrence is the aetiological factor in the minor defects of "small umbilical" or "amniotic" herniae. Is it of more than passing interest that in deficiencies of the anterior abdominal wall the offending tissue is the mesenchyme of the early embryonic rim—the site of fusion of primary and secondary mesoderm?

SUMMARY

1. The umbilical cord is formed by the growth of lateral tissue plates which extend on each side from the allantoic stalk to the septum transversum.

2. The umbilical cord coelom enlarges by the extension of the endothelial lining into the large spaces in the cord tissue formed by the confluence of smaller spaces.

3. The temporary physiological herniation of a part of the gut is a concomitant of the extra-embryonic position of the yolk sac which itself is an expression of a modification to meet nutritional requirements.

4. The observations support the suggestion already offered by Frazer that the return of the gut into the abdominal cavity is dependent on a "negative" intra-abdominal pressure.

5. The obliteration of the umbilical cord coelom is effected by a proliferation of the denser fibrous tissue which forms an encircling collar at the embryonic attachment of the cord.

6. This circular zone of mesodermal condensation contributes to the linea alba above and below, and to the rectus sheaths on each side of the umbilicus.

7. Some fault of the junctional mesoderm, i.e. the tissue at the embryonic rim where there is union of primary and secondary mesoderm, is responsible for congenital herniation which results either from maldevelopment of the cord itself or from deficiency of the neighbouring parietes.

I have again to record indebtedness to my Chief, Prof. Blair, for his continued guidance and help. Dr P. Bacsich was consulted on several questions of histological interpretation.

The 42 mm. embryo was kindly given on loan by Prof. T. Nicol and Dr Gladstone, of King's College, London.

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EXPLANATION OF PLATES I–V

PLATE I

Figs. 1, 2. Photomicrographs of sections of embryo 2·6 mm. × *circa* 60.
Figs. 3, 4. Photomicrographs of sections of embryo 4·5 mm. × *circa* 60.
A. = amnion. *L.T.* = lateral tissue plate. *U.* = umbilical vein. *C.* = coelom. Description in text.

PLATE II

Figs. 5, 6. Photomicrographs of section of embryo 7 mm. × *circa* 18.
Fig. 7. Photomicrograph of section of distal part of umbilical cord of embryo 7 mm. × *circa* 85.
Fig. 8. Photomicrograph of section of embryo 8·5 mm. × *circa* 18.
U.C. = umbilical cord coelom. *I.* = intercoelomic septum. *D.* = distal slit-like portion of umbilical cord coelom. *S.* = space in umbilical cord. *U.* = umbilical vein. Description in text.

PLATE III

Fig. 9. Photomicrograph of section of embryo 12·5 mm. × *circa* 12.
Fig. 10. Photomicrograph of section of embryo 14 mm. × *circa* 12.
Fig. 11. Photomicrograph of section of embryo 16·1 mm. × *circa* 11.
Fig. 12. Photomicrograph of section of embryo 23 mm. × *circa* 11.
U. = umbilical vein. *I.* = intercoelomic septum with denser mesoderm extending into cord. *M.* = myotomic downgrowth. Description in text.

PLATE IV

Fig. 13. Photomicrograph of section of embryo 40 mm. × *circa* 6.
Fig. 14. Photomicrograph of section of embryo 42 mm. × *circa* 7.
Fig. 15. Photomicrograph of section of umbilical cord coelom of embryo 42 mm. Site indicated by arrow in fig. 14. × *circa* 75.
Fig. 16. Photomicrograph of endothelial lining of umbilical cord coelom of embryo 42 mm. × *circa* 225.
E. = denser mesoderm extending into cord "pillars" and continuous with rectus sheath. *U.C.* = umbilical cord coelom. *L.* = degenerating endothelial cells. Description in text.

PLATE V

Figs. 17–20. Photomicrographs of sections of the ventral body wall of embryo 60 mm. × *circa* 6.
B. = linea alba formed from rectus sheath. *E.* = denser tissue of midventral line extending into cord. *F.* = denser tissue on superficial aspect of allantoic vessels. Description in text.

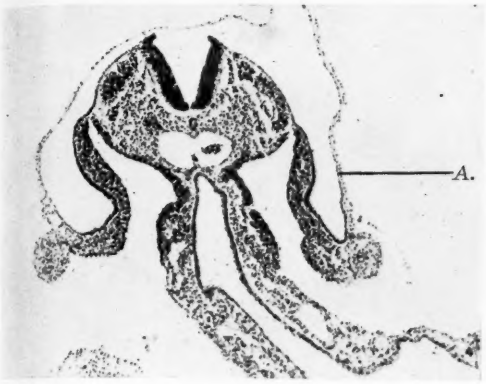
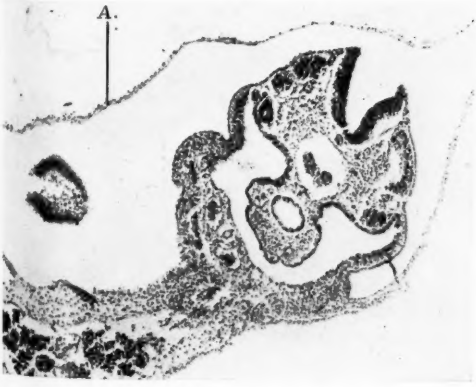


Fig. 1.

Fig. 3.



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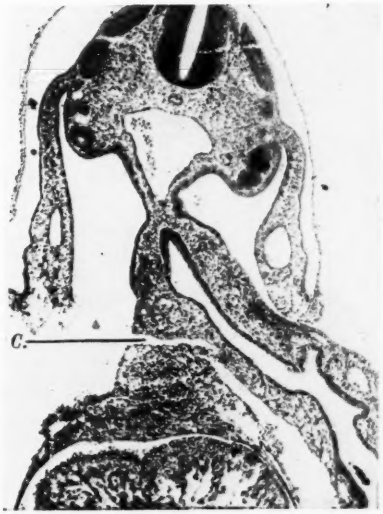

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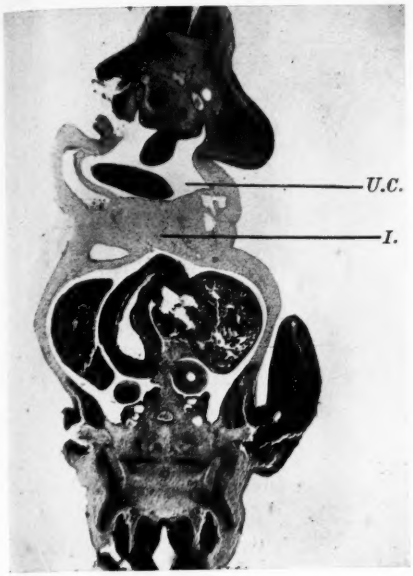

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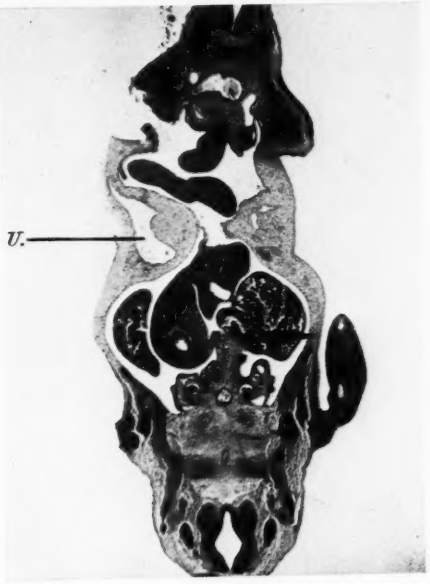

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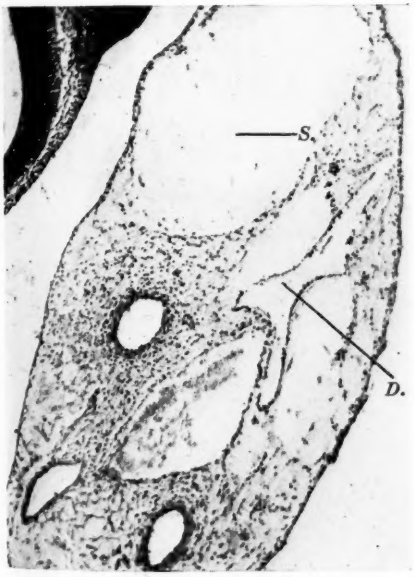

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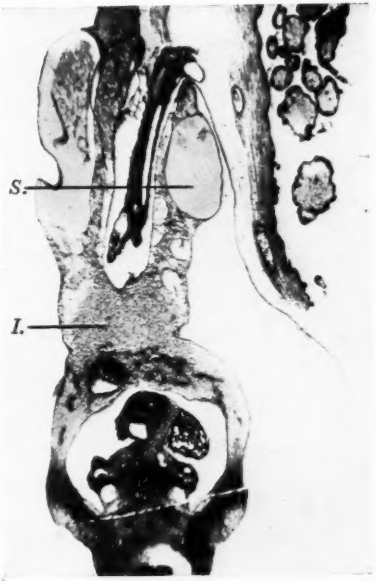

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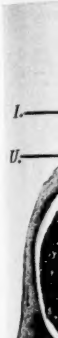
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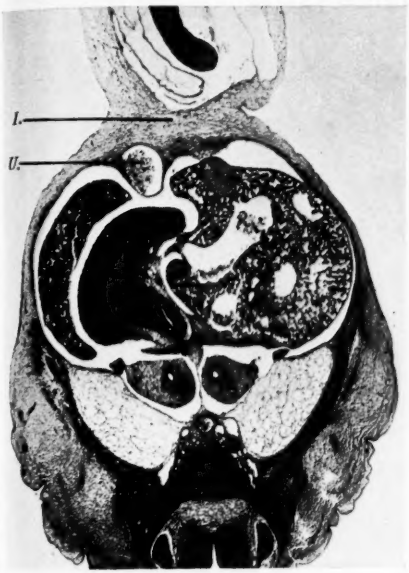

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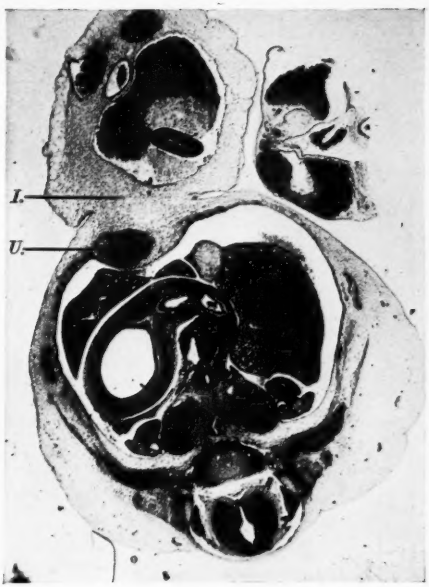

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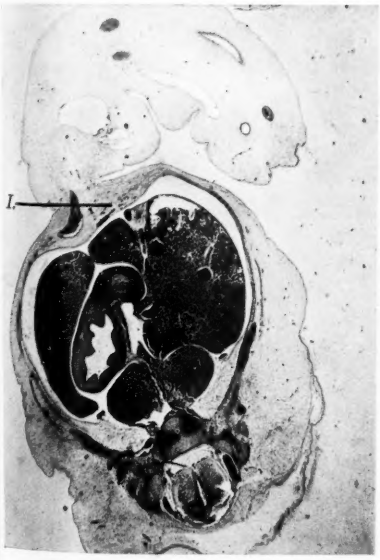

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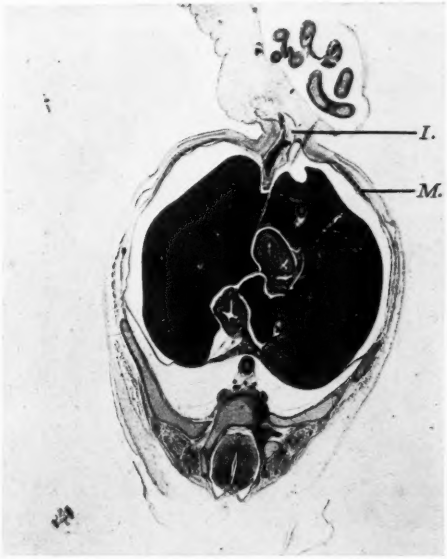

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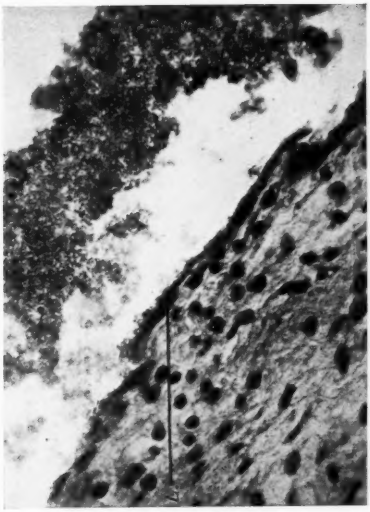

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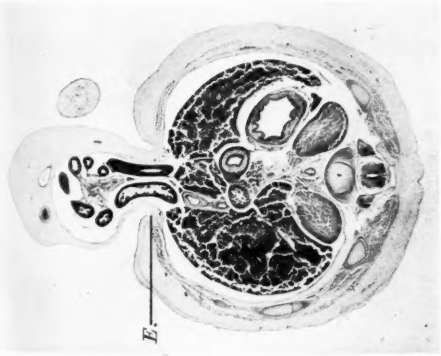

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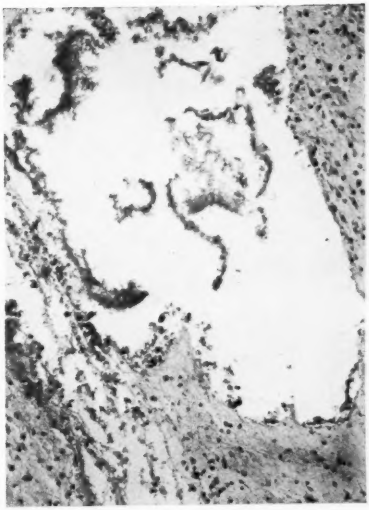

Fig. 15.

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Fig. 18.

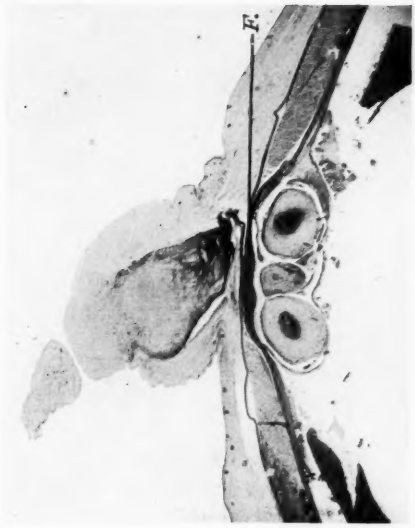

Fig. 20.

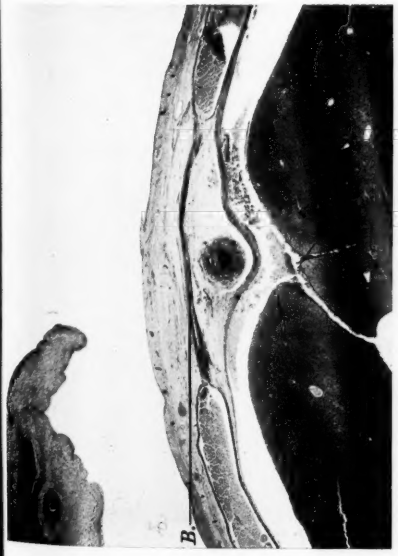

Fig. 17.

Fig. 19.

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MYRMECOPHAGA JUBATA: AN EMBRYO WITH PLACENTA

By ELDRED W. WALLS, B.Sc., M.B., Ch.B.

Lecturer on Anatomy in the University of Glasgow

Myrmecophaga jubata is one species of the family Myrmecophagidae, which belongs to the order Edentata.

The embryo and placenta here described were obtained by Agar in 1907 in South America. The material was preserved in bulk (Pl. I, fig. 1) till 1920, when it was sectioned by Miss Marshall, and a description of the embryo given by her.

The placenta has not hitherto been described.

THE EMBRYO

The length of the embryo is 10 mm. and bears a close resemblance in external appearance to a human embryo of the same size. Points of differentiation are, first, the extreme elongation of the tail end, and secondly, the incomplete closure of the neural tube in the caudal half of the embryo. The abdominal wall is completely closed, there being no evidence of a yolk sac. The limb rudiments are distinctly seen, and myotomes are present from slightly tailwards of the forelimbs. Three pharyngeal arches are formed, and in this connexion I would make further comment. In her description Miss Marshall mentioned an asymmetrical development of the pharynx. I am of the opinion that this is more apparent than actual owing to the obliquity at which the head end has been cut. This obliquity of section is not very obvious until examination is made of the reconstructed head region, using a model made at a high magnification. This method of examination demonstrates the essential bilateral symmetry of the head end of the embryo.

The three main subdivisions of the brain are well marked, and from the forebrain which shows a very slight indication of the two hemispheres, the stalked optic vesicles can be clearly seen growing outwards towards the lens vesicles which are fully formed. In connexion with the hindbrain the otocyst has sunk in from the surface ectoderm, and growing from it is the ductus endolymphaticus. There is no trace of cerebellum.

The alimentary canal shows a simple arrangement; the oesophagus passes into a dilated portion of the tube destined to be the stomach, and from there to the anus the gut has a very direct course except for a loop into the umbilical cord coelome. The cloaca, widely expanded, is still separated from the exterior and has passing into it the Wolffian ducts from which the ureteric buds can just be seen coming off, and arising from it the allantois, which will be described more fully later.

THE PLACENTA

In the *Journal of Anatomy and Physiology* of 1876, Turner described in a very general way two specimens of the placenta of the Cape anteater (*Orycteropus*). The material used was far from fresh, and as a result Turner was unable to reach any definite conclusions as to the true nature of this animal's placentation.

In Miss Marshall's paper no mention of the placenta was made, other than that its shape was discoidal, which fact can be observed in Pl. I, fig. 1.

In 1928 Wislocki gave details of the placentation in the two-toed anteater, *Cyclops didactylus*; he described it as discoidal in shape, villous with trabeculae, and belonging to group 4 of Grosser's classification.

Becher in 1931 described the placenta of a species very closely related to *Myrmecophaga jubata*, namely *Tamandua tetradactyla*. Here again the placenta was haemochorial with villi and trabeculae.

Thus, although the placentae of closely allied species have been described, no account of the placentation of *M. jubata* has yet been given.

The placenta of Myrmecophaga jubata

The placenta, which is well formed at this stage of gestation, is discoidal in form, and large relative to the embryo, measuring 35 mm. in diameter, and increasing from a depth of 5 mm. at the centre to 9 mm. at the periphery. Examination of the different tissues entering into its structure reveals three distinct areas. Two of these are foetal in origin and form the greater bulk of the organ; the third is formed from maternal tissue, and constitutes a very limited portion on the uterine side of the placenta. The foetal tissue can be differentiated into two parts which, although at first sight seeming quite distinct prove in reality to be simply different degrees of modification of the original trophoblast. This tissue, i.e. the trophoblast, has on the foetal side of the placenta formed a large villous area. Centrally the villi are very large and show numerous branching processes, but as they are followed towards the circumference of the placenta they become much smaller and less branched. An arrangement of them into clusters or cotyledons can be established for the most part, but the maternal side of the placenta does not show subdivision into lobules correspondingly. Whether this character has been present and by this stage become less marked, or whether it will later appear, cannot of course be stated from the examination of a single specimen.

The trophoblastic tissue which has not proceeded to form villi lies to the maternal side of these processes forming about half the thickness of the organ centrally and practically its full thickness marginally. Strands of this undifferentiated trophoblastic tissue, which could be described as trabecular trophoblast, pass between the villi, and many of these are intimately fused with the villi.

The most important relationship of the villi, however, is that which they

bear to the maternal blood. As they pass towards the uterus they are bathed directly in this medium, no tissue of any kind intervening. From what has been said before, the undifferentiated trophoblast must also bear this relationship to the maternal blood. Throughout the foetal portion of the placenta, therefore, maternal blood lies in direct contact with the foetal tissue. In certain places it is much greater in amount, namely, surrounding the villi and the peripheral part of the trabecular trophoblast. The latter, it should be stated, is much more loosely arranged than the more centrally placed trophoblast in immediate relationship to the villous tree.

Detailed structure of villi and trabecular trophoblast

The villi. Surrounding each villus and forming its outer wall is a ring of protoplasm in which is set a row of cells. These cells vary in shape in different villi, but the majority are either cubical or sausage-shaped; in a few instances they are spindle-shaped. In all cases, however, they are lying connected to each other by the limiting protoplasm giving an appearance highly suggestive of a syncytium were it not for the fact that the cell outlines can be distinguished. Each cell has a well-marked nucleus and numerous granules scattered throughout its cytoplasm, and quite a number of cells can be seen to be in a state of active division. Within the outer wall is a vascular core of mesoderm, and in a few villi a central blood vessel is beginning to make its appearance. In certain of the villi the outer wall does not seem to have such a simple structure as that described above. In these villi there seems to be present a second layer of cells subjacent to those set in the protoplasmic ring. It is possible that this inner layer is simply formed of mesodermal cells arranged in an orderly way, but if not then the two layers might be styled plasmoditrophoblast and cytotrophoblast. Yet another possibility is that the inner layer being mesodermal the outer is a primitive type of covering which later in phylogeny will give rise to both the cyto- and plasmoditrophoblast.

The trabecular trophoblast. This undifferentiated foetal tissue is extremely cellular. The cells, which are round in shape, are closely packed together and are very well stained, the nuclei and cell granules standing out sharply. Spaces do exist, however, in this tissue despite its great cellularity, and so maternal blood can permeate it throughout its extent. When close examination is made of the places at which the villi and trabecular trophoblast are in contact it is found that a precise demarcation of where trophoblast becomes villus is impossible. The rounded trophoblast cells gradually become drawn out as the villus is approached and then merge into its wall as the characteristic sausage-shaped cells.

It is seen, then, that in the placenta of *Myrmecophaga jubata* there are separating the maternal and foetal blood streams (*a*) villus wall, possibly formed of two layers, (*b*) foetal mesoderm, and that, being so, the placenta can be styled haemochorial, the same as occurs in *Cyclops didactylus* and *Tamandua tetradactyla*, both members of the family Myrmecophagidae. An interesting

point, however, is that all species of this order have not the same type of placentation, a fact which is very well brought forth when reference is made to Wislocki's description of the minute structure of the placenta in *Bradypus*. Here it is found that the placenta is labyrinthine, transitional in character between the syndesmochorial and endothelial types of Grosser, and that separating the maternal and foetal blood streams there are:

(*a*) Endothelium of maternal blood vessels.

(*b*) Fibrous tissue surrounding the blood vessels, of maternal origin.

(*c*) Trophoblast which, as maturity approaches, becomes very thinned out and may disappear over large areas.

(*d*) Epithelioid cells originally mesodermal in character which arrange themselves between the foetal stroma and the trophoblast.

I have mentioned above that the placenta of *Myrmecophaga jubata* can be classified as haemochorial, but from its detailed structure it would appear to embody in its character features of both types of haemochorial placentation, i.e. villous and labyrinthine.

The maternal tissue or decidua is found as a thin layer on the outer side of the placenta. In the centre of the organ it is rather less than 1 mm. thick, and when followed towards the circumference becomes very much thinner, and in many places actually is deficient. The cells of the decidua are much less numerous than in the underlying trophoblast, and further points of difference are these:

(1) The size of the cells is greater.

(2) Their shape is variable, some being round, others spindle-shaped, and a number of quite irregular outline.

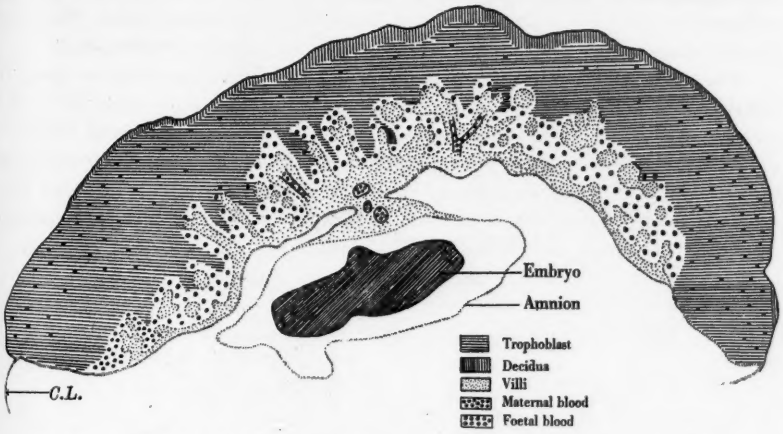
(3) The cells stain poorly, both cytoplasm and nuclei, and in fact they give the appearance of being in a state of hyaline degeneration.

There is a very slight space between the decidua and the trophoblast in which very few cells are present; from their character they seem to have migrated from both these layers. It is possible that the space here described is an artefact produced in the preparation of the sections.

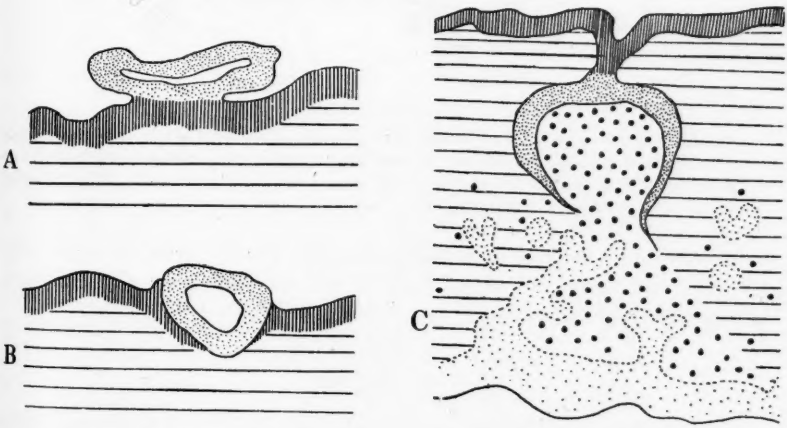
The foetal membranes. Identification of the individual foetal membranes of this embryo and placenta is difficult. The amnion is still in position and completely surrounds the embryo. At the margin of the placenta small tags of chorion laeve remain but no trace of decidua capsularis or decidua vera can be found.

The blood supply to the placenta. Maternal blood reaches the placenta by means of a single large artery which, at first running deep to the uterine mucosa, gradually passes into the substance of the placenta. In its course the decidual tissue of the organ is very closely applied to its wall, and as these two tissues, i.e. decidua and blood vessel wall, are very similar histologically, exact delimitation is in places very difficult. The artery continues deeply into the placenta and terminates by opening widely around the central villi. From this source the maternal blood oozes to all parts of the placenta, bathing the villi

and percolating through the undifferentiated trophoblast, but it should be noted that it never penetrates the decidua. The return of blood from the



Text-fig. 1. Diagram of the complete placenta and membranes. *C.L.* = chorion laeve.



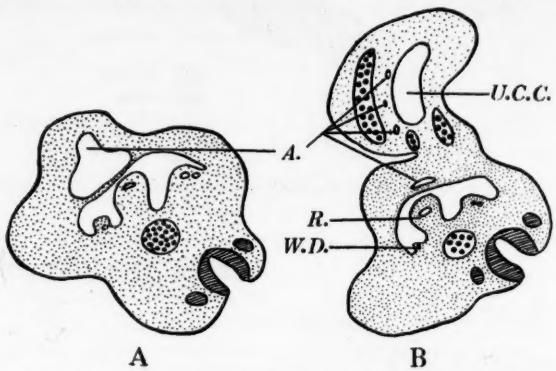
Text-fig. 2. Diagram of blood supply to the placenta. The different tissues are represented as in Text-fig. 1. A, the maternal artery is here lying on the uterine surface of the decidua. B, the vessel is now sinking through the decidua into the underlying trophoblast. C, lastly it is seen opening widely into the intervillous space.

placenta is effected by means of a companion vein which, although smaller than the artery, is nevertheless easily followed out.

The umbilical cord. This is a large thick structure which passes almost direct from embryo to placenta which it joins a short distance from its centre.

In composition it is very similar to the human cord, the loose tissue forming it containing a number of structures. These are, first, three large blood vessels, two arteries and a vein; secondly, the umbilical cord coelome into which a loop of bowel is passing, and lastly, the remnant of the allantoic canal. The cord by this stage, at any rate, has not developed any degree of spiral twist.

The allantois. In the region of the urogenital sinus the allantoic canal is widely expanded and is clearly seen opening into that sinus anterior to the rectum. However, it rapidly tapers off as it passes upwards, and where it enters the umbilical cord is a very small canal. Tracing it along the cord it is found that it ends about halfway, quite failing to reach the placenta. This vestigial state of the allantoic canal corresponds to that met with in the armadillo (Newman & Paterson) at a corresponding stage of development.



Text-fig. 3. Diagram of two sections through the allantois. A, showing widely dilated allantois at entrance to cloaca. B, the allantois is seen much thinned out passing upwards in the abdominal wall and into the umbilical cord. *R.* = rectum, *A.* = allantoic canal, *W.D.* = Wolffian duct, *U.C.C.* = umbilical cord coelome.

In concluding the description of the placenta, I would draw attention to the very poor vascularity of the villi, coupled with an established placental form, as compared with a human ovum of similar embryo stage.

SUMMARY

1. The embryo of *Myrmecophaga jubata* is at the 10 mm. stage very similar in external form to a human embryo of the same size. Points of difference are the extreme elongation of the tail end and the non-closure of the caudal half of the neural tube.

2. The placenta is a well-developed discoidal structure which can be classified as allantoic deciduate in type. It is faintly cotyledonary in character, and its detailed structure is haemochorial, i.e. it belongs to group 4 of Grosser's classification. It is neither purely villous nor labyrinthine, but rather a composite type of placenta.

Fig. 1.

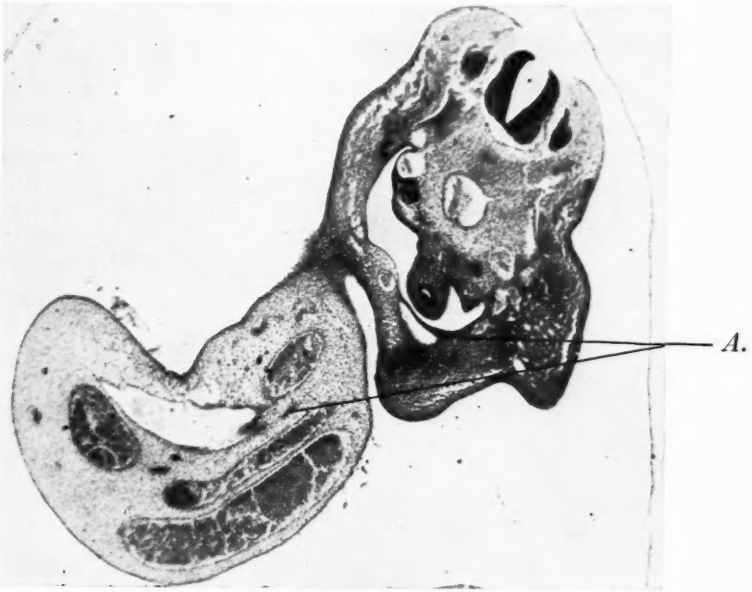


Fig. 2.

WALLS—*Myrmecophaga jubata*: an Embryo with Placenta

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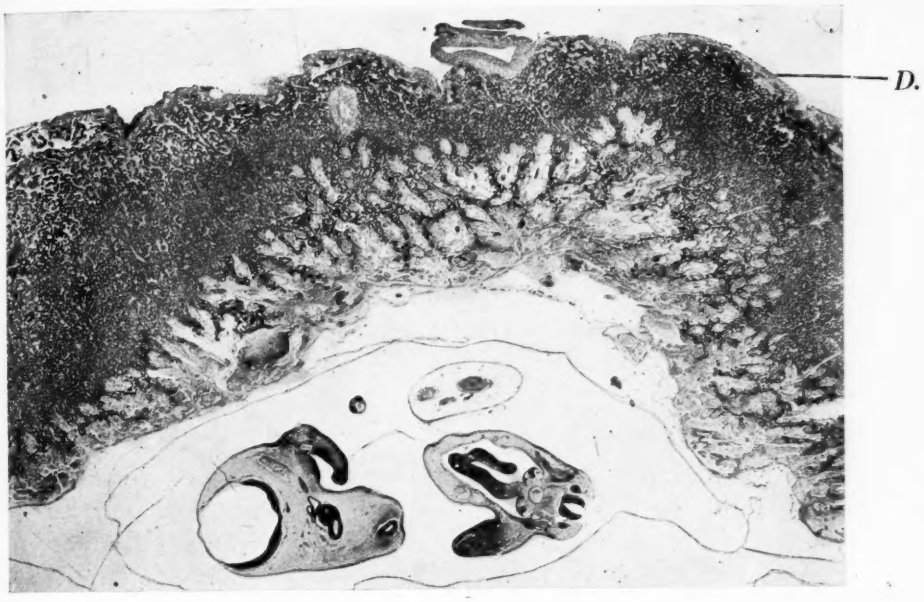
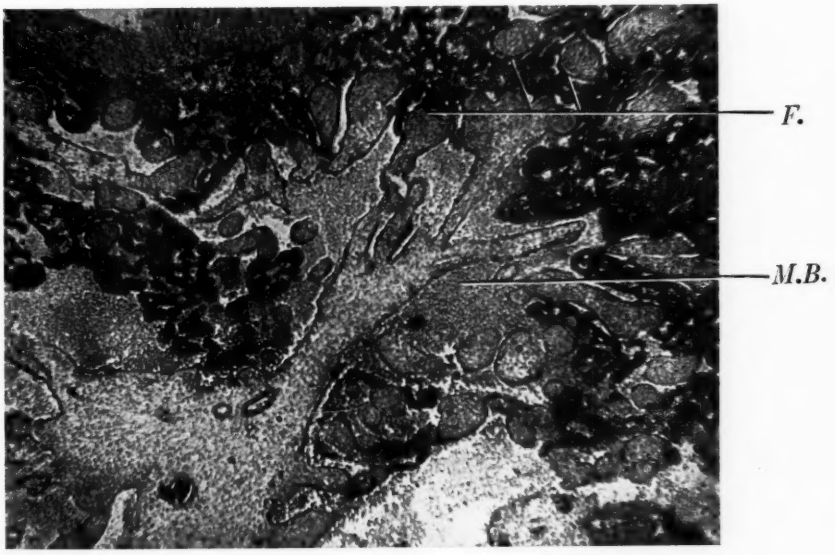


Fig. 3.



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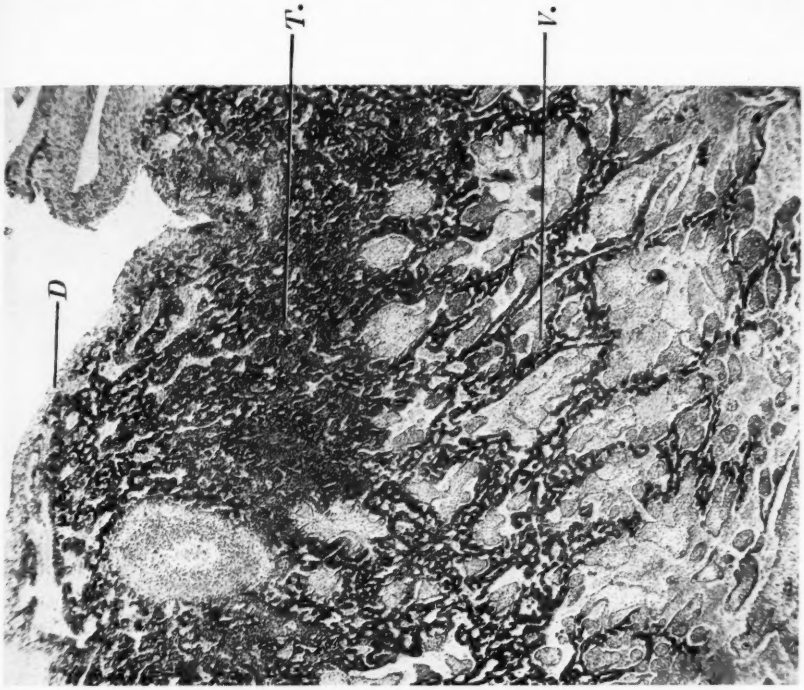

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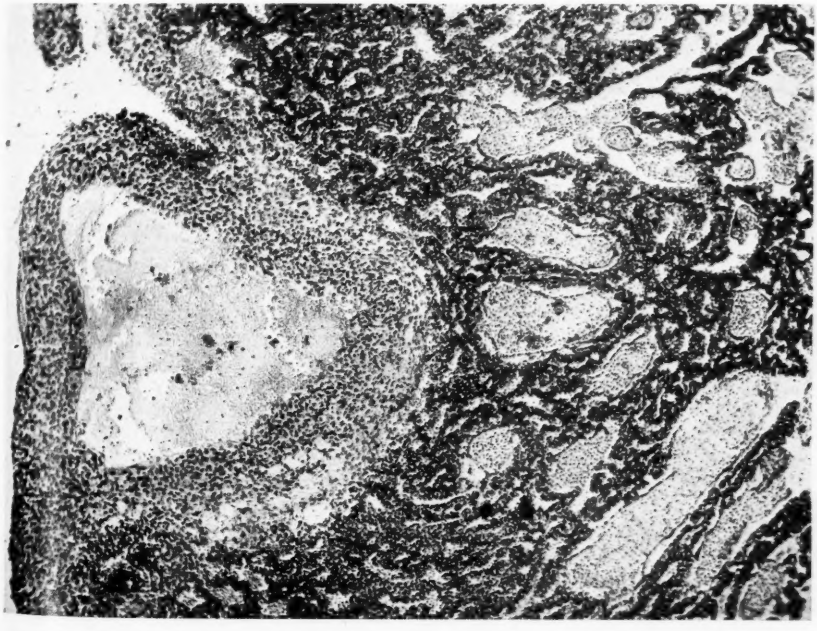

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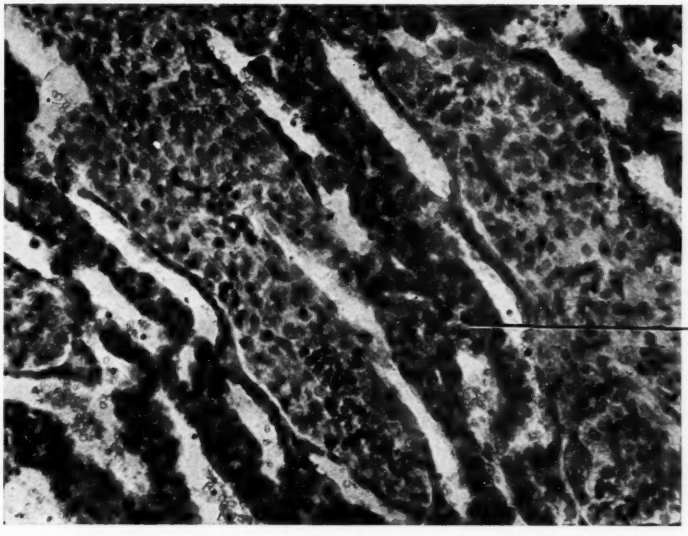

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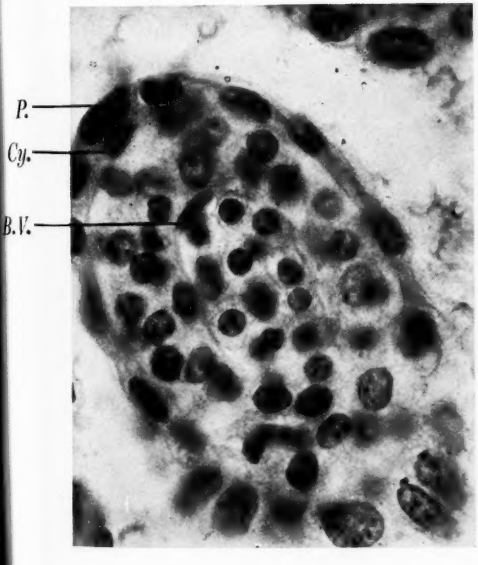

Fig. 8.

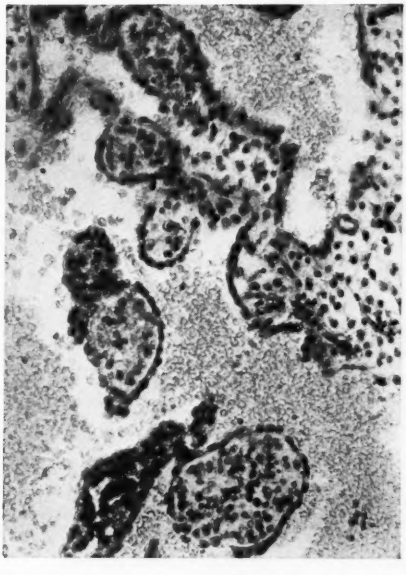

Fig. 9.

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ACKNOWLEDGEMENTS

I desire to express my indebtedness to the late Dr Norman Maclaren, to Prof. J. P. Hill of University College, London, and to my chief, Prof. D. M. Blair, for their helpful advice and criticism, and to Mr George Marshall for his skill in preparing photomicrographs 1, 2, 4, 7 and 8. For photomicrographs 3, 5 and 6 I am indebted to Mr Pittock of University College, London.

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EXPLANATION OF PLATES I–IV

Plate I

Fig. 1. Photograph of material before sectioning. The general characters of the embryo can be seen. Note the elongated tail passing up the right side of the head.

Fig. 2. Transverse section of lower abdomen and umbilical cord showing allantoic canal (*A.*) in abdominal wall and in cord. ×40.

Plate II

Fig. 3. Low power photograph to show full thickness of placenta. The decidua (*D.*) can be seen as a very thin layer on the uterine surface of the placenta.

Fig. 4. Section through cluster of villi. Note villi surrounded by maternal blood (*M.B.*), and the fusion (*F.*) of the villi and trophoblast. ×166 *circa*.

Plate III

Fig. 5. The large maternal artery is here seen sinking into the placenta. The intimacy with which its wall blends with the decidua is well shown.

Fig. 6. The three zones of the placenta are clearly shown in this section. *D.* = decidua, *T.* = undifferentiated trophoblast, *V.* = villous area.

Plate IV

Fig. 7. To show trophoblastic strands (*T.S.*) passing between the villi. The gradual change in character of the trophoblast cells as they merge into the villous wall is well seen.

Fig. 8. Transverse section of villus. Plasmoditrophoblast (*P.*) and cytotrophoblast (*Cy.*) are seen surrounding a central blood vessel (*B.V.*). ×300.

Fig. 9. Transverse section and longitudinal section of villi lying free in maternal blood. ×125.

OBSERVATIONS ON THE NEURAL CREST OF A TEN-SOMITE HUMAN EMBRYO

By J. S. BAXTER and J. D. BOYD

In the course of a study of a ten-somite human embryo in a good state of preservation we observed an unusual arrangement of those neural crest cells which form the acoustico-facial primordium. This led us to investigate the arrangement and distribution of the whole of the neural crest, and, as there are few detailed accounts of the neural crest in human somite embryos, we present our results as a separate contribution.

The embryo was obtained by laparotomy from a case of tubal gestation. The clinical history suggests that, if ovulation occurred about the mid-point of the menstrual cycle, the conceptional age of the embryo is approximately 28 days.[1] When received in the laboratory the intact chorionic vesicle had been fixed in formalin (of unknown strength) for several days. In its longest axis the vesicle measured 14 mm., in its shortest 12 mm. Having been dissected from the main part of the chorion, the embryo, with the connecting stalk and the adjacent chorionic wall, was stained in bulk with alum cochineal, dehydrated by the drop method, imbedded in paraffin and cut transversely at 8 μ into a complete series of sections. The sections were then stained on the slide with orange G.

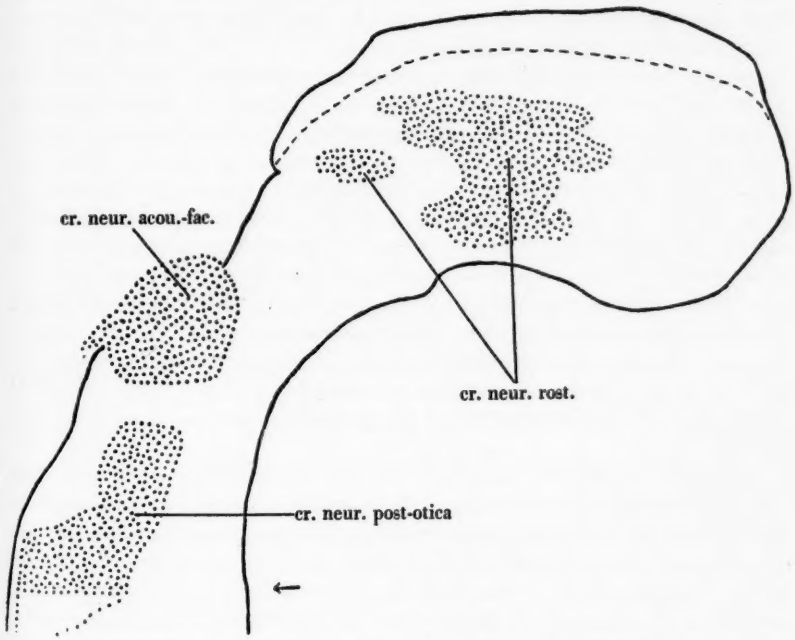
A number of reconstructions of this embryo have been made. Those of interest for the purpose of this description are a wax-plate model of the external form of the embryo, and a millboard-wax model (according to the method of Green, 1937), of the cranial portion of the nervous system and its associated neural crest primordia.

The sectioned embryo, and the reconstructions, show that the neural folds have united from a point somewhat cranial to the otic placodes to the level of the seventh somite. Here the closure of the neural tube is discontinuous, and the caudal extremity of the neural plate is a thickened, flattened area of columnar cells continuous laterally with the somatic ectoderm. The appearance of this caudal portion of the nervous system is very similar to that presented by other human embryos at a comparable stage of development. The cranial extremities of the neural folds, however, are not widely separated and thus contrast with the condition usually found at this stage. The anterior neuropore is, then, a narrow, deep cleft (Pl. I, figs. 1, 2); and, in our opinion, it is quite possible that this appearance may be ascribed to undue shrinkage of the

[1] We are indebted to Dr H. T. Laycock of Addenbrooke's Hospital, Cambridge, for the opportunity of studying this specimen. Details of the clinical history of the patient from whom the embryo was obtained will be given in a subsequent contribution.

embryo during fixation. There is a certain degree of asymmetry in the state of development of the two sides of the neural primordium, the right side of the central nervous system being more advanced than the left. This asymmetry has been described in a number of human somite embryos (cp. Bartelmez & Evans, 1926).

There are three regions in our embryo in which neural crest cells are found in relation with the developing nervous system (see Text-fig. 1). The most



Text-fig. 1. Geometric projection of the right profile view of a reconstruction of the cephalic portion of the nervous system. The various primordia of the neural crest are shown. The hatched line indicates the margin of the left neural fold. The arrow shows the cranial limit of the first somite.

anterior of these regions is at the level where the cranial flexure is most pronounced, and here, on either side of the open neural folds, there is a discrete mass of neural crest cells. The right one is better developed and will be described first.

It is, obviously, the *crista neuralis rostralis* of Bartelmez & Evans, or the *craniale Kopfganglienleiste* of Veit (1919). Although, caudally, there is a small isolated portion of this neural crest material, we do not think that a distinct subdivision into a "pars optica" and a "pars trigeminalis" is present. The neural crest cells do, however, extend forwards to the dorsal edge of the optic

sulcus and, possibly, the aggregation of cells should be regarded as a compound of a *crista neuralis rostralis* (associated with the trigeminal area and the Gasserian ganglion) and a *crista neuralis prosencephali* (from the dorsal edge of the optic primordium). If this interpretation is correct then the term *primordium opto-cristale* of Bartelmez & Evans might be used for the anterior part of this portion of the neural crest. We incline to the opinion, however, which has been expressed by Adelmann (1925), that the crista neuralis prosencephali should be regarded with extreme caution. Adelmann was unable to find any trace of such a portion of the neural crest in a large number of rat embryos of the somite stage; he states (p. 52), "absence of such a neural crest from at least a major portion of the forebrain seems to be a general condition". Schulte & Tilney (1915) were also unable to find neural crest cells arising from the prosencephalon during the early development of the central nervous system of the cat. Indeed, these investigators were so impressed by the absence of neural crest cells in the region of the forebrain that they suggested the retention of these cells in the wall of the neural tube to form an element additional to the alar and basal laminae. We are unable to find any evidence for such a retention of alleged neural crest cells in the anterior portions of the neural plates, but we have the impression that the cephalic portion of our crista neuralis rostralis is not of prosencephalic origin but is an extension forwards of the main part of the mass which is related primarily to the anterior part of the rhombencephalon and the mesencephalon. It is, probably, that portion of the crest which will be concerned with the development of the ophthalmic division of the trigeminal nerve.

The cells constituting this right anterior neural crest mass do not show continuity with the neural plate or the somatic ectoderm. In this character (which may be due to the same shrinkage process which has caused the approximation of the two halves of the open neural plate in this region) they differ from the corresponding cells in the ten-somite embryo described by Corner (1929), for he both described (p. 90), and figured, cells passing to this mass from "the inner surface of the neural epithelium and the neuro-somatic junction" of the entire midbrain region and the rostral part of the rhombencephalon. We have been unable to find a trigeminal placode in our embryo so the question of a contribution from this source to the neural crest does not arise.

There are distinct differences between the neural crest cells and the surrounding mesenchyme, but it is impossible to be definite in the identification of the cells in the transition zone between the two groups. The bulk cochineal stain is, as in Corner's specimen, not well adapted to show cytoplasmic differences. We feel quite certain, however, that the neural crest material extends towards, and merges with, the mesenchyme of the first branchial arch, particularly with that portion of the arch which will later form the maxillary process. The existence of this mingling of neural crest cells with the surrounding mesenchyme does not, of course, constitute evidence for the derivation of mesenchyme from neural crest cells. On this point our material does not

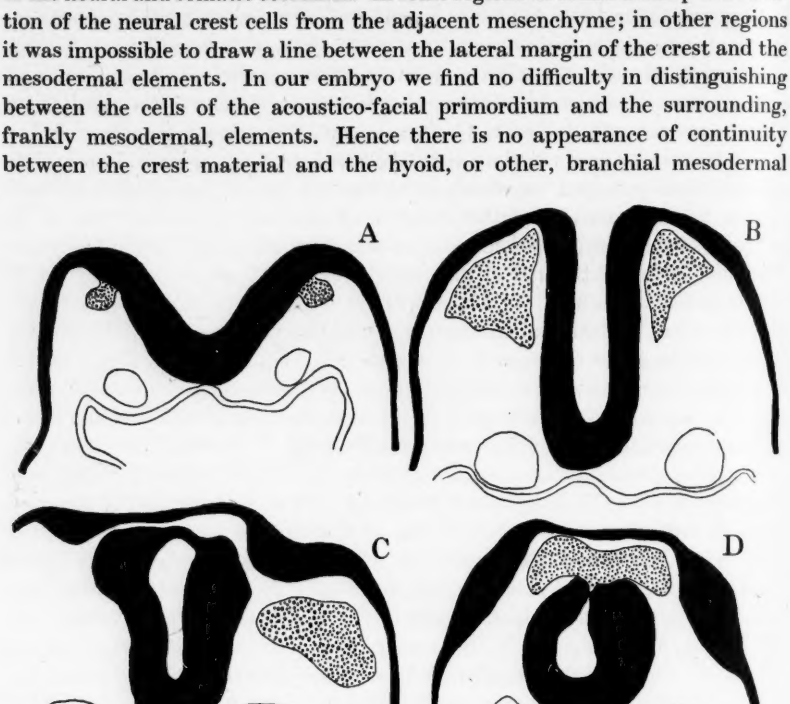
allow of any definite statement and, indeed, we doubt the value of any contributions to this problem which are based on description alone, particularly on the study of a single embryo or a very small series of embryos.

On the left side of the presumptive midbrain region there is a mass of neural crest cells which in position and relations is similar to the right crista neuralis rostralis (Pl. I, figs. 1, 2). It is, however, very much smaller than the corresponding mass of the right side. We correlate this difference in size with the relatively retarded development of the left side of the central nervous system in the head end of this embryo. As on the right side, there is no evidence of the migration of neural crest cells from the edge of the neural plate. The smaller size of this left crista neuralis rostralis probably accounts for its better delimitation and the absence of an appearance, on this side of the embryo, of an extension of the crest material into the branchial mesenchyme.

Caudal to these two masses of neural crest cells there is a region of the developing nervous system which has no such cells related to it. A short distance behind the anterior limit of fusion of the neural folds there is found the next aggregation of neural crest cells (Pl. I, figs. 1, 2). This portion consists of a large mass of cells situated dorsal to the neural tube and intimately connected with it (see Pl. I, fig. 3 and Pl. II, fig. 7). The cephalic part of this mass forms a continuum across the mid-line of the embryo with no trace of subdivision into right and left halves; but, when this mass of neural crest cells is traced caudally it separates into two, bilaterally symmetrical, horn-like processes which come to lie on either side of the dorsal portion of the neural tube (Pl. II, fig. 5). This group is the *primordium acoustico-facialis* (Bartelmez & Evans), or Veit's *caudale Kopfganglienleiste*, and is that portion of the neural crest associated with the hyoid arch and the acoustico-facial nervous complex. It is situated, as the figures show, in close relation with the otic placodes, but there is no appearance which would suggest a contribution of placodal cells to the crest material. The neural crest cells can easily be differentiated from the cells of the adjacent neural tube, but most of the sections through this region show an appearance as if crest cells are being budded off from the line of apposition, in the median plane, of the two halves of the neural plate. The appearance, in fact, suggests that complete dorsal fusion of the neural folds has not yet taken place (see Pl. II, fig. 7), although they are completely separated from the overlying, continuous, somatic ectoderm by the relatively huge mass of neural crest cells. This acoustico-facial primordium is so large that it produces a definite protuberance upon the dorsal aspect of the embryo.

The form taken by the acoustico-facial neural crest mass in this embryo is very different from that seen in Corner's ten-somite human embryo (cp. Text-fig. 2B). In his specimen the anterior limit of fusion of the neural folds has not extended so far forward as in our embryo. It reaches only to the level of the acoustico-facial neural crest primordium which, here, is represented by bilateral and entirely separate cell aggregations; these, according to Corner's description and microphotographs, seem to stream out of the angle of junction

of the neural and somatic ectoderm. In some regions he found a sharp demarcation of the neural crest cells from the adjacent mesenchyme; in other regions it was impossible to draw a line between the lateral margin of the crest and the mesodermal elements. In our embryo we find no difficulty in distinguishing between the cells of the acoustico-facial primordium and the surrounding, frankly mesodermal, elements. Hence there is no appearance of continuity between the crest material and the hyoid, or other, branchial mesodermal



Text-fig. 2. Diagram to show the arrangement of the acoustico-facial neural crest primordia in certain human embryos. A. Payne embryo of seven somites. B. Corner embryo (ten somites). C. Heuser embryo (fourteen somites). D. Baxter-Boyd embryo (ten somites). Ectoderm, neural folds and tube indicated in solid black; acoustico-facial neural crest material stippled; endoderm and dorsal aortae outlined. (For explanation see text.)

condensations. Other human embryos of the somite period in which the neural crests have been studied seem to correspond with Corner's description of the arrangement of the acoustico-facial neural crest elements. Thus in Payne's (1925) embryo of seven somites (Text-fig. 2A), and Veit's embryo of eight somites, there are two, quite separate, acoustico-facial primordia; and Heuser (1930), in his fourteen-somite specimen, describes bilateral acoustico-facial primordia in an embryo with complete closure of the neural tube in the definitive hindbrain region (Text-fig. 2C).

There is, then, a marked difference between the acoustico-facial neural crest primordium in our embryo and the same region in comparable human embryos. Our specimen may, of course, be pathological; tubal gestations are

notoriously prone to show abnormalities of development. We are inclined, however, to regard it as showing no more than a variation of the usual neural crest cell pattern, for, if further development had occurred, it is quite possible that the mid-line mass would have separated into the typical, bilateral, acoustico-facial primordia. Indeed in its caudal portion there is already present such a separation (Pl. II, fig. 5) and the appearance here is very similar to that shown by other human somite embryos. The neural crest is primarily a derivative of the lateral part of the neural plate and whether it arises by proliferation from the lateral margins of the neural folds or from the roof of the closed neural tube is dependent upon the time at which the neural folds fuse dorsally. With delayed closure the tendency will be for the crest cells to arise from the edges of the neural folds and bilateral primordia will result. When closure is early the cells will take their origin from the dorsal portion of the fused folds and will, therefore, form a temporary mid-line mass of neural crest material. Lenhossek (1891) described both these modes of origin in the human embryo. Since the crest cells arise from the junctional zone between the somatic and neural ectoderm it is not surprising that, for a short time, immediately after closure of the neural tube, some crest cells are found dorsal to the line of closure. Raven (1932) has published figures of the development of the neural crests in the Axolotl which show a mid-line arrangement of a considerable extent of the crest material dorsal to the closed neural tube and a number of other workers (cp. Brachet, 1935) have drawn attention to this temporary arrangement in other vertebrates.[1] In our embryo, however, cells belonging to the neural crest are found in the dorsal mid-line only at the level of the acoustico-facial primordium; none are found at more caudal levels although closure of the neural tube has occurred as far back as the seventh somite. We conclude, therefore, that in this specimen the cause of the unusual arrangement of the acoustico-facial neural crest cells was either precocious closure of the neural tube at this level only, or a delay in the time of appearance of the neural crest cells. As other ten-somite human embryos show a less degree of closure of the neural tube than our embryo, we think the former explanation to be more correct; and if this be accepted, then the variation is easily understandable and is of no morphological significance, involving, merely, a time relationship.

[1] When this paper was presented at the Summer Meeting of the Anatomical Society, 1938, Prof. J. P. Hill showed a section through the acoustico-facial crest of a sparrow embryo in which the distribution of the crest cells was very similar to that shown by our human embryo. He kindly drew our attention to the work of Belogolowy (*Zur Entwicklung der Kopfnerven der Vögel*, Moscou, 1910), who figures sections through the neural crest of chick embryos in which there is a mid-line arrangement of the crest cells. Belogolowy writes: "Charakteristisch für diesen Nerv (acousticofacialis) ist eine erhalten bleibende Zellgruppe der dorsalen Seite des Neuralrohres." Unlike our specimen, however, the acoustico-facial primordium in the chick appears to be connected as much with the ectoderm as with the neural tube. It would seem that the time relations between closure of the neural folds and differentiation of the crest cells differ in bird and mammal. Our specimen in this respect resembles the avian pattern, but development has proceeded too far to show the connexion between the acoustico-facial primordium and the overlying ectoderm which is completely separated from it.

We have been unable to find any evidence in our embryo for a contribution to the acoustico-facial neural crest primordium from the otic placode. The histological state of the material, however, and the fact that we are dealing with a single embryo prevents us from making a significant statement on this possibility. We use the term *acoustico-facial primordium* as it is the customary one employed in the literature to designate this portion of the neural crest. Prof. J. P. Hill (personal communication) has expressed the opinion that it should be called the facial primordium, as he regards the acoustic ganglion to be largely, if not entirely, of placodal origin. This opinion is supported by Brachet (1907), Landacre (1921) and Knouff (1927), all of whom worked on amphibian material, and, more recently by van Campenhout (1935), who worked on the pig embryo. On the other hand, Adelmann (1925) states that the VIIIth ganglion is a derivative of the common acoustico-facial mass, owing its origin entirely to neural crest proliferation. Our embryo is too young to permit of a definite statement on this point, but obviously if the acoustico-facial complex is partly of crest and partly of placodal origin the term acoustico-facial primordium is still justifiable, whereas if the VIIIth ganglion is completely a derivative of the placode then *facial primordium* would be a more suitable term for this part of the neural crest.

Caudal to the single mass of acoustico-facial neural crest cells there is a small portion of the neural tube which has no crest cells related to it. The next subdivision of the neural crest takes the form of two masses of cells, one on either side of the pre-somite region of the developing hindbrain (Text-fig. 1). These masses are about equal in size; each is related to the dorso-lateral aspect of its own half of the neural tube, but there is no appearance of continuity of the crest cells across the middle line. Corner, in his description of his ten-somite embryo, uses the term "occipito-spinal neural crest" for all the crest material found behind the acoustico-facial primordium. We think, however, that this group of crest cells posterior to the acoustico-facial mass and anterior to the region of the somites should be distinguished as a separate portion and should be called, as Bartelmez & Evans designate it, the *crista neuralis post-otica*. It is that portion of the crest which is related to the post-hyoidean visceral arches and which is concerned in the development of the glossopharyngeal and vagus nerves. The surface ectoderm of our embryo at this level shows, on either side dorsally, definite thickenings, and although they are not so circumscribed as those described by Corner, we presume that they correspond to his "Discus IX and X". We are unable to find any trace of a contribution, at this stage, to the crest cells from these areas of thickened ectoderm.

Behind the post-otic neural crest is found what we regard as the true occipito-spinal portion of the crest (Pl. II, fig. 6). This extends, on either side, from the region of the first somite, where it is more or less continuous with the crista post-otica, to about the level of the sixth somite. It is not a continuous column as it shows frequent small gaps. The gaps, however, do not have a segmental arrangement and there is no evidence of neural crest cells being

more, or less, opposite to the somites, or the intersomitic spaces. At no point are the cells very numerous and, while there is no definite evidence for their derivation from the neural tube, the histological appearances are often very suggestive of a connexion between the crest cells and the dorsal portion of the closed tube. Caudal to the sixth somite there is no trace of a neural crest (Pl. I, fig. 4) the angle between the neural fold and the somatic ectoderm being completely free of cells. In this hinder region of the developing nervous system we are unable to find any cells which we can take to be forerunners of neural crest cells and there is no sign of activity in the adjacent ectoderm which would suggest that they may be derived from this source.

That part of the occipito-spinal neural crest which lies opposite the first three somites is probably concerned with the development of the neurones and sheath cells which, in later stages, are connected with the hypoglossal and accessory nerves. Enough is not yet known about the developmental history of this region for any statement to be made on the precise boundary of this "occipital" portion of the crest. The importance of establishing an embryological basis for the interpretation of the accessory nerve and the dorsal root ganglia of the hypoglossal nerve, however, makes some definition, even one which is only of topographical significance, essential. In the absence of better criteria the first three somites appear to be suitable as a first approximation to the establishment of precise boundaries for this portion of the neural crest. In our sections this part of the crest and, indeed, the more caudal portion, shows no evidence for a continuity with the mesenchyme, the crest cells being always well-defined groups with no appearance of transition into the surrounding mesodermal elements. The caudal part of the crest opposite to the fourth, fifth and sixth somites is presumably the rudiment of the dorsal root ganglia of the upper cervical region but, as has already been stated, it shows no evidence of the segmentation so characteristic of later stages. In general appearance and in the arrangement of its cells this "spinal" portion of the neural crest resembles very closely the "occipital" portion with which it is directly continuous.

SUMMARY

The neural crest primordia of a ten-somite human embryo are described. It is shown that they form an interrupted column of cells on either side of the developing nervous system and reaching from the midbrain region to the level of the sixth somite. In cephalo-caudal sequence the primordia are (1) crista neuralis rostralis (concerned in the development of the trigeminal nerve); (2) crista neuralis acoustico-facialis (facial and, possibly, acoustic nerves); (3) crista neuralis post-otica (glossopharyngeal and vagus nerves); (4) crista neuralis occipitalis (related to the first three somites); (5) crista neuralis spinalis (related to the fourth, fifth and sixth somites). The last three groups of neural crest cells form an irregular continuum.

The crista acoustico-facialis of this embryo is peculiar in that it forms a

midline mass which, anteriorly, shows no sign of separation into right and left components. It is suggested that this variation is within the normal range and is the result of precocious closure of the neural tube in this region.

From a study of this single embryo no definite statement can be made on the precise mode of origin or the fate of the neural crest cells.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Reconstruction of the future brain region of the embryo viewed from above and behind. In order better to show the dark masses of neural crest material against the background they have been outlined with white dots. The asymmetry of the neural folds and the corresponding asymmetry of the cristae neuralis rostralis is to be noted. The form of the crista neuralis acoustico-facialis is well seen.

Fig. 2. The same reconstruction viewed from in front; the neural crest primordia are again outlined with a white dotted line. The crista neuralis acoustico-facialis in this view overlaps somewhat the crista neuralis post-otica, but the boundary between the two is indicated.

Fig. 3. Microphotograph of a section through the cranial part of the acoustico-facial primordium of the embryo (× 250). The single mass of cells of this primordium lying dorsal to the neural tube and fused with it in the midline is to be noted. There is no apparent connexion between this primordium and the overlying somatic ectoderm.

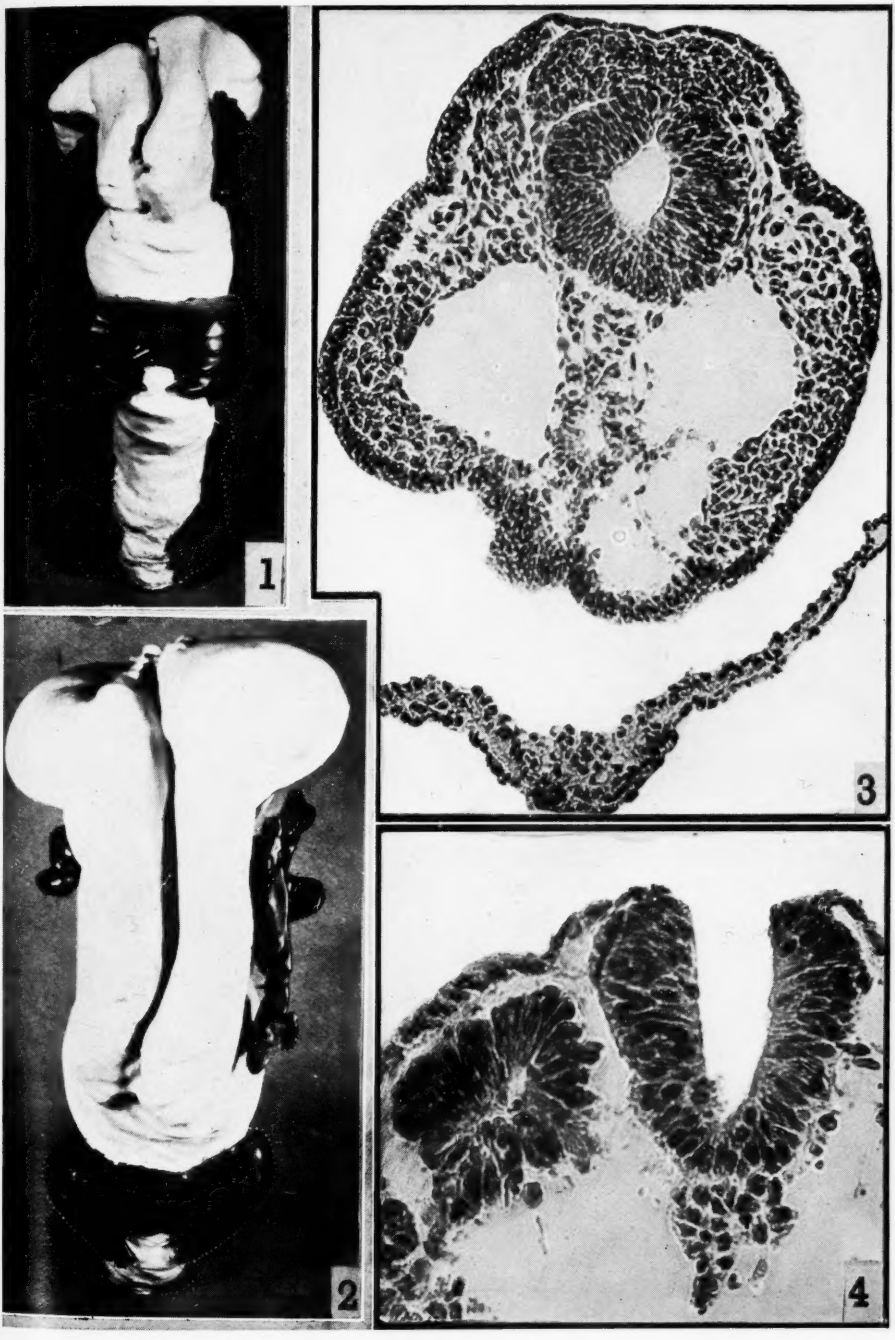
Fig. 4. Microphotograph of a section at the level of the eighth somite (× 500). Here there are no neural crest cells in the re-entrant angle between the neural and somatic ectoderm. The neural folds at this level are quite separate.

PLATE II

Fig. 5. Microphotograph of a section passing through the caudal part of the acoustico-facial primordium (× 500). Here the primordium is seen as bilaterally symmetrical masses of cells lying dorso-lateral to the neural tube (cp. figs. 1 and 7). The otic placodes are well shown.

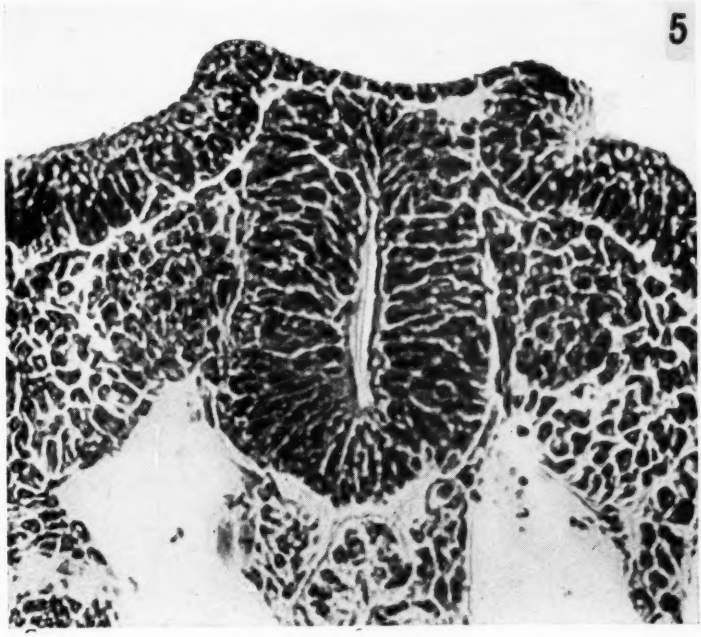
Fig. 6. Microphotograph of section at the level of the first somite (× 500). Neural crest cells of the occipito-spinal group are to be seen in the angle between the neural tube and the somatic ectoderm.

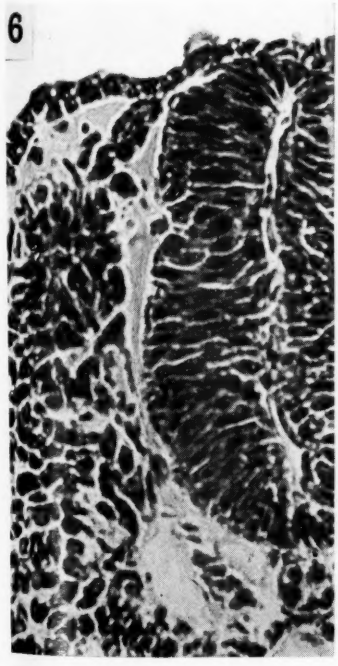
Fig. 7. Microphotograph of a section through the cranial part of the acoustico-facial neural crest primordium (× 500). There is an appearance here as if cells are being budded off dorsally from the region of apposition of the neural folds to join the mass of crest cells. The neural folds are seen not to be completely fused.

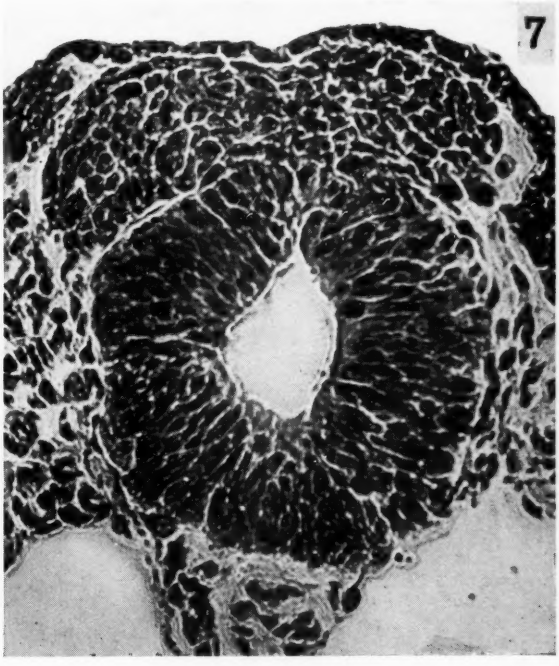


BAXTER AND BOYD—THE NEURAL CREST OF A TEN-SOMITE HUMAN EMBRYO

UNIV. OF MICH.







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THE BRAIN OF A GIRL, AGED 12, SAID TO BE THAT OF AN ABORIGINAL AUSTRALIAN

By JOSEPH L. SHELLSHEAR
University of Sydney

Whilst preparing a group of fifty European brains, collected in Sydney, for examination and comparison with the brains of the aboriginal Australian and Chinese, I was struck by the very unusual grouping of primitive features shown in brain 19 of the collected series. This grouping was in harmony with my description of the aboriginal Australian (1937); and I remarked at the time that, either the brain was that of an aboriginal, or there is no cerebral criterion of race. I had not read the labels on the jars, having no doubt from the source of supply that the brains were all of European origin. Reference to this jar, however, revealed: "*Female, age* 12, *said to be the brain of an Australian aboriginal whether of full blood not known.* 23 *Dec.* 1936."

The unprejudiced recognition of the racial features in this brain, the value of its morphological characters, the fact that it adequately summarizes my extensive account of the aboriginal brain, and the comparative rarity of Australian material are all valid reasons for its publication.

The figures illustrating the sulcal pattern have been made from photographs taken with a telescopic lens. The sulci were drawn in on the photographs in such a way that, where one sulcus meets another but is clearly separate, it is drawn as a separate sulcus; and, where it is deeply embedded but separate, its separate nature is indicated by two dots at the point of separation.

THE GENERAL FORM OF THE BRAIN

Though features of form are difficult to describe, an intensive study of the endocranial casts of living and extinct races, taken in conjunction with knowledge acquired from other sources, emphasizes their importance. The form of the brain gives, as it were, a bird's eye view of the lobes together with their relative degrees of development, and tends to counteract the more contracted view obtained in the detailed study of the sulci with their confusing variability.

It is interesting that the features of form which characterize this brain should be just those features which Gratiolet, and afterwards Marshall, should have specially selected in the brain of the Bushman as significant at a time when that significance was so little known.

Brain 19 is well fixed, but there is some slight distortion. The dorsal surface is somewhat flattened and the occipital lobes diverge somewhat from each other; nevertheless, the general form can be readily appreciated.

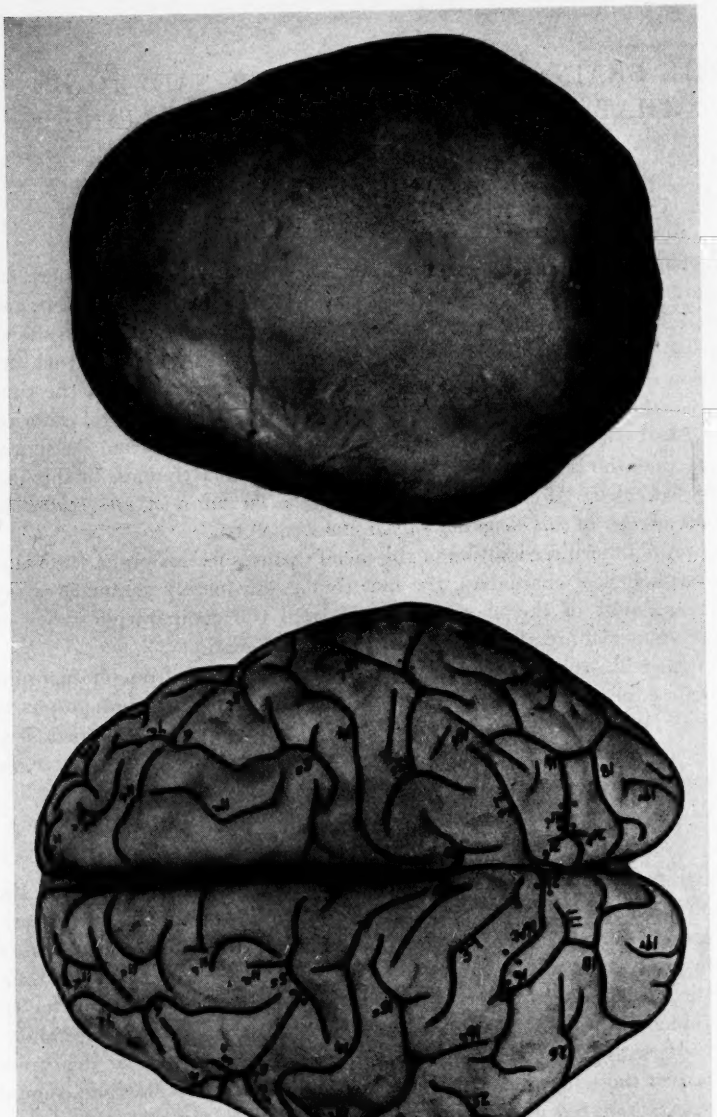

Fig. 2. Photograph of the dorsal surface of the endocranial cast of the skull of an Australian child.

Fig. 1. Photograph of the dorsal surface of the brain. The sulci are drawn in ink. Where there is a deep separation of the sulci it is indicated by two dots.

Seen from above (Fig. 1) the form of the brain presents those primitive features which Elliot Smith (1928) has so frequently described as significant in the brain of early man; and which I have emphasized in the brain of the Bushwoman described by Marshall. The area of greatest width is formed by the parieto-temporal eminence which lies immediately posterior to the bifurcation of the fissure of Sylvius, and practically corresponds with the gyrus supramarginalis. The term parieto-temporal eminence is here used to define the general enlargement of the brain in the region where the posterior part of the temporal lobe and the inferior parietal region meet. It is formed by the fusion of the two localized areas of growth, named by Elliot Smith (1928) the temporal and parietal eminences, which are separate in many of the earlier members of the human family. In the most primitive forms, *Sinanthropus*, *Pithecanthropus* and *Eoanthropus*, the temporal eminence is the most lateral point of the hemisphere, the parietal eminence being ill-developed. In *Homo rhodesiensis* the two eminences are still distinguishable from one another, but the parietal eminence has developed and expanded to a much greater extent than in the lower forms. With this greater expansion of the parietal region the vertex has grown higher; but there are definite areas of ill-fitting associated with it. In *Homo neanderthalensis* the two eminences have merged into a comparatively wide rounded area producing the parieto-temporal eminence. Such also is the state of development in the brain under consideration which is in marked contrast with the brain of the European where the expansion of the parietal region has progressed beyond the limits of a localizable parieto-temporal eminence into an extensive filling of the whole parietal lobe forcing the area striata from the lateral surface.

From the parieto-temporal eminence in the brain under discussion the posterior part of the general outline, seen from above, tapers sharply to a rounded occipital pole composed almost entirely of striate cortex well defined by an occipital operculum. The general contour, apart from the sulcal pattern which will be examined separately, indicates the very localized nature of the expansion of the supramarginal region, and the lack of development of the angular and postparietal regions. The form of the posterior part of the brain is similar to that of Marshall's Bushwoman (Fig. 19); and that this is not a mere coincidence is obvious if only a cursory examination is made of the sulcal pattern, which is instructively primitive and similar in the two forms.

Anterior to the parieto-temporal eminence, the general outline seen from above again bears a striking resemblance to that of the Bushwoman. The outline curves forwards over the inferior postcentral and central regions as far as the level of inferior precentral sulcus where a somewhat abrupt angle is formed as the contour passes to the frontal lobe. The angulation of the contour at this point is clearly shown in this brain, but is not so marked as it is in the endocranial casts of *Pithecanthropus* and *Sinanthropus* and in the brain of Marshall's Bushwoman. The diminution in width of the brain immediately anterior to the

central region is brought about by the precentral depression of Elliot Smith and the general ill-development of the prefrontal areas.

In discussing these features of form in the brain of the Bushwoman, where all the sutures were closed (1934, p. 5), the suggestion was made that the small size of the frontal lobes may be secondary to changes in the skull consequent upon age. This can now be definitely ruled out on account of the age of this girl, and also on account of the description of the brain of the Bushman by Isidore Slome (1932).

Fig. 2 is a photograph of the endocranial cast of a skull of an aboriginal child from the Queensland Museum. It shows the same form features as

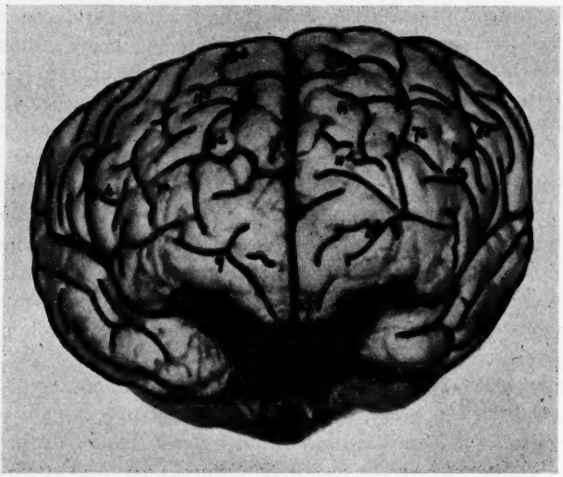

Fig. 3. Photograph of the frontal aspect of the brain.

brain 19 and is here used to correct any misconception conveyed by the slight degree of distortion in the brain under consideration.

From the front (Fig. 3), the narrowness of the frontal lobes and the slope backwards to the parieto-temporal eminence are well illustrated. In the brains of the European (British Isles) and Chinese the width across the level of the frontal caps of Broca obscures in great measure the precentral and central sulci when seen from the anterior aspect; whereas in this brain all the sulci as far posteriorly as the postcentral sulcus are visible from the front. Anterior to Broca's cap the orbital margin of the frontal lobe is highly arched and ill-filled, and ends anteriorly in a prominent rostral, or frontal, keel. Not only are all the features again closely paralleled in the brain of Marshall's Bushwoman, but also the sulcal pattern in both forms has a corresponding degree of simplicity.

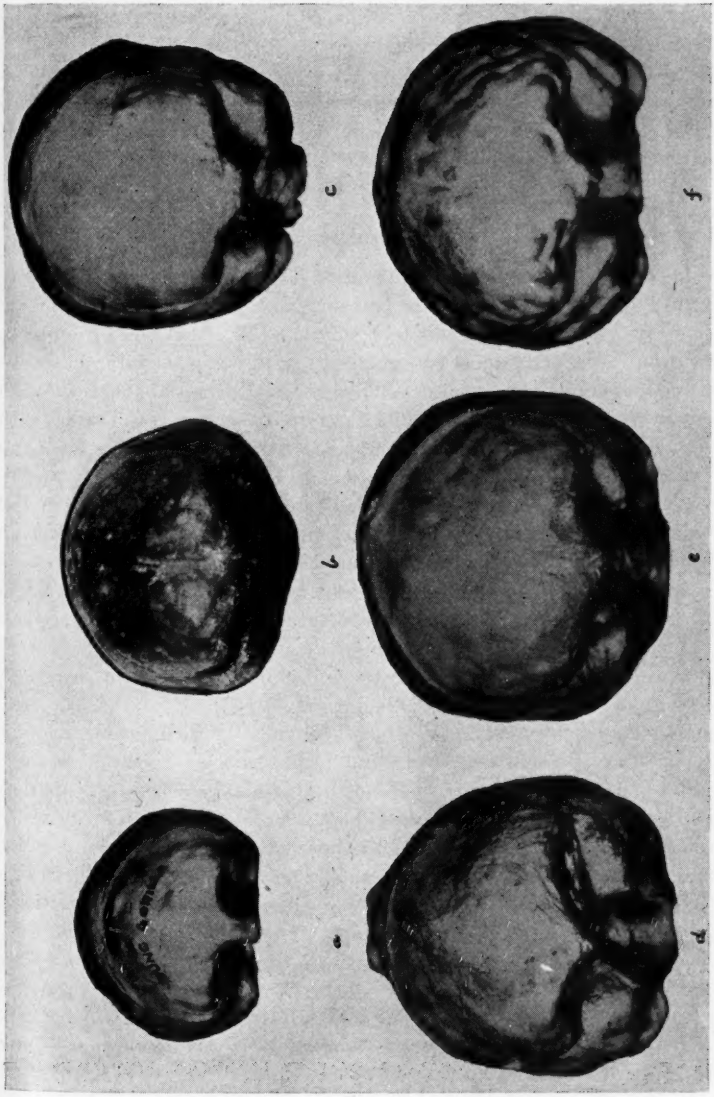

Fig. 4. Photographs of the frontal aspect of the endocranial casts of (*a*) gorilla, (*b*) *Sinanthropus*, (*c*) an Australian child, (*d*) an Australian adult, (*e*) a Chinese, (*f*) a European.

The degree of development of the orbital margin is approximately intermediate between that seen in *Sinanthropus* and a modern Chinese or European. This is clearly shown in Fig. 4, showing respectively photographs of the frontal aspect of the endocranial casts of the gorilla, *Sinanthropus*, an aboriginal child, an aboriginal adult, a Chinese and a European.

Viewed from below (Fig. 11), the width of the parieto-temporal region and the narrowness of the frontal lobes are apparent. This view also demonstrates the somewhat pointed appearance of the temporal poles and the relatively large extent of exposure of the orbital surfaces of the frontal lobes.

The sulcal pattern of the brain

The lateral occipital region (Figs. 5 and 6). There is a remarkable degree of symmetry in the sulcal pattern of the two sides, not only in the occipital region but elsewhere. The occipital, or lunate, operculum (18) stands out prominently. Lying in front of it, and almost concentrically with it, on the lateral aspect of the brain is a complete and well developed anterior occipital sulcus (26). The form of the operculum and of the sulci lying on its surface is the same on the two sides. It has the appearance of an arcade hanging down from the paroccipital sulcus (16), so that the major convexity lies above. Retraction of the operculum discloses the bottom of the opercular sulcus at least an inch below the point where the paroccipital sulcus and operculum meet. In Fig. 6 the sulci hidden by the opercula are represented by broken lines. On both sides the sulcus paroccipitalis passes practically to the bottom of the opercular sulcus; but there is a very narrow submerged gyrus separating them. Thus a transverse occipital sulcus cannot be recognized. At first sight the small sulcus 17? might be taken for the inner arm of the sulcus occipitalis transversus; but it is more probably the incisura parieto-occipitalis. The depth of the opercular sulcus is as great as I have seen in any human brain; the whole of the operculum is a thin tongue of reduplicated cortex thrown forward over the occipital lobe. The prelunate sulcus (18*p*) passes forwards into the parietal region on both sides, arising at a somewhat lower level on the left side.

Lying on the posterior surface of the operculum is a long vertical sulcus (19*v*) extending from a point 5 mm. from the posterior lip of the sulcus right down to the occipital pole itself. This is the sulcus retrocalcarinus verticalis.

LEGENDS TO FIGURES 5–8

Fig. 5. Photograph of the posterior aspect of the brain. (Note that sulcus 16*h* is shown as apparently joining , but this is only because of the aspect from which the photo is taken.)

Fig. 6. Same as Fig. 5 with the sulci hidden under the occipital operculum diagrammatically indicated.

Fig. 7. Photograph of the lateral aspect of the right hemisphere.

Fig. 8. Photograph of the lateral aspect of the left hemisphere.

Figs. 7 and 8

Figs. 5 and 6

It is an intrastriate sulcus and illustrates the vertical type of folding of the striate cortex. An inferior polar sulcus surrounds the lower end of the sulcus retrocalcarinus verticalis on both sides. It is present on the tentorial surface of the hemisphere.

The type of opercular sulcus seen in this brain provides an essential clue to the understanding of the homologies of the sulci in the occipital lobe, and strengthens the arguments already advanced (1937, p. 301) regarding the identification of the occipital sulci. These arguments tended to harmonize many conflicting views regarding the homology of such sulci as the paramesial, transverse occipital, superior and inferior polar, lunate etc. As the various questions were there discussed the reader is referred to the original paper.

As the sulcus retrocalcarinus turns from the medial to the lateral surface of the hemisphere it has the appearance of conforming to the vertical disposition of the area striata by turning upwards parallel with the sulcus retrocalcarinus verticalis on the surface of the occipital operculum. The remarkable symmetry of the two sides is seen even in the detail of this disposition of the posterior end of the sulcus retrocalcarinus (20). There is no terminal branching of the sulcus retrocalcarinus.

The anterior occipital sulcus (26) (Figs. 5, 7 and 8). This sulcus extends from the post-parietal lobule, close to the paroccipital sulcus (16) above, to the region of the preoccipital notch below. It bounds the preoccipital cortex anteriorly. The derivation of this sulcus from the parallel sulcus has been described in a previous communication (1927); and there it was shown how in the development of the inferior parietal lobule it becomes displaced backwards and retains its association with the visual area. This close relationship explains why a complete arcade is more commonly found in primitive forms, and naturally, in these forms, it occurs as a sulcus concentric with the occipital operculum. In higher forms the upper and lower elements may become separated as an expression of the increasing complexity of pattern. On both sides of this brain the two elements are confluent to form a complete arcade which is continuous at its midpoint with the parallel sulcus. Another interesting feature in this brain is the indication of an apparent line of growth placed in the axis of the visuo-auditory band of Elliot Smith. The anterior occipital sulcus is joined to the parallel sulcus by an annectant sulcus (x) to which the lower branch of the fissure of Sylvius points anteriorly and the prelunate sulcus posteriorly. This is more clearly seen on the left side. This stress line is apparent in the brain of the higher apes. On the left side the anterior occipital sulcus is deeply confluent inferiorly with the occipito-temporal sulcus.

LEGENDS TO FIGURES 9–11

Fig. 9. Photograph of the medial surface of the right hemisphere.

Fig. 10. Photograph of the medial surface of the left hemisphere.

Fig. 11. Photograph of the ventral aspect with the cerebellum removed to show the tentoria surface. (The cerebellum is, however, indicated by an interrupted line.)

Fig. 11

Figs. 9 and 10

The medial surface of the occipital lobe

The parieto-occipital fissure (21) (Figs. 9 and 10). On both sides this fissure extends from the calcarine sulcus below to a point well on the dorsal surface above (Fig. 1). At first sight the condition appears to conform with those types in which the expansions of the cuneus and precuneus have produced anterior and posterior opercula of the parieto-occipital fossa resulting in a continuous parieto-occipital fissure with complete burying of the arcus intercuneatus. The condition here however is in reality primitive. On the left side the apparent parieto-occipital fissure is vertical in direction and the lower half has a backwardly directed convexity indicating a localized expansion of the lower part of the precuneus. The upper part of the precuneus, bounded in front by the sulcus cinguli (10) and behind by the sulcus limitans praecunei (21*l*), is ill-developed and narrow, the sulci of the precuneus are simple and indicate absence of expansion, the paracalcarine sulcus (21*p*) is clearly defined, and finally, the arcus intercuneatus is exposed on the surface between 21*l* and 21*p*. These facts prove that the apparent parieto-occipital fissure is really the sulcus limitans praecunei. There is no incisura parieto-occipitalis on the medial surface; but the small sulcus (17 ?) associated with the paroccipital sulcus on the dorsal surface probably represents it. On the dorso-medial border of the hemisphere the upper end of the sulcus limitans praecunei is separated from the inner end of the sulcus parietalis transversus (Fig. 1, 16*pt*) by a submerged gyrus. But even apart from the ill-development of the precuneus and the exposed condition of the arcus intercuneatus with the exposed paracalcarine sulcus, the contracted parieto-occipital arcade points clearly to the primitive nature of the medial occipital region.

On the right side the arcus intercuneatus is almost completely hidden; the process of infolding of the parieto-occipital fossa is at a stage when the upper ends of the paracalcarine sulcus (21*p*) and the sulcus limitans praecunei (21*l*) are exposed at their upper ends. In Fig. 1 the apparent continuation of the parieto-occipital fissure into the paroccipital sulcus is shown. On opening up this fissure however, the incisura parieto-occipitalis (21*i*) is seen between the diverging sulci as a relatively shallow sulcus separated from the sulci medial and lateral to it. A comparison of the two sides as seen from the dorsal aspect (Fig. 1) shows that on the right side the parieto-occipital fissure is at a more posterior level and the parieto-occipital arcade is more diminutive. On comparing the two sides from the medial aspects (Figs. 9 and 10) it is apparent that the difference in form of the parieto-occipital fissure is due to the greater development of the upper half of the precuneus on the right side with more richly folded precuneal and subparietal sulci. Nevertheless the folding is simple when compared with higher forms.

The retrocalcarine sulcus (20) presents the same form and relations on the two sides. Anteriorly it is directly continuous with the anterior calcarine sulcus (20*a*), and where they meet with the parieto-occipital fissure the gyrus cunei,

commonly seen in the Australian brain, is absent; posteriorly the retrocalcarine sulcus passes horizontally backwards almost to the occipital pole where it turns upwards, as it were sliding on the upper part of the sulcus retrocalcarinus verticalis (19*v*), thus emphasizing the vertical folding of the area striata on the lateral surface. This turning upwards of the two retrocalcarine sulci gives point to my contention (1937, p. 301) that we must seek further for the homologies of the occipital operculum of the apes than in the laterally placed sulcus lunatus of man. In the extensive operculum of the Chimpanzee there are elements which in part homologize with the transverse occipital, the superior polar, the inferior polar, the lunate and even the inferior occipital sulci from within outwards as they are found in the human brain. In the brain under consideration the dominant part seems to be played by the superior polar and lunate sulci.

The relatively small size of the cuneus, the form of the cuneal sulci and of the sulci limiting the area striata superiorly and inferiorly are shown on Figs. 9 and 10.

The sulci of the lateral surface of the parietal region (Figs. 7 and 8)

The fissure of Sylvius (2). On both sides this fissure is completely closed so that there is no exposure of the island of Reil. The anterior horizontal (2*h*) and anterior ascending (2*a*) branches are disposed in a U-shaped pattern. The anterior horizontal arm is slightly higher on the left side than it is on the right, otherwise there is nothing of moment in the arrangement. The main horizontal arm of the fissure of Sylvius passes backwards and ends by dividing into ascending and descending branches immediately posterior to the transverse postcentral sulcus (15*i*). The distance between the lower end of the sulcus centralis and the point of bifurcation of the fissure of Sylvius is slightly greater on the left side than it is on the right; but the difference is not so much as it is in many other Australian brains (1937, p. 345).

The parallel sulcus and its associated branches

The form of the parallel sulcus (23) in this brain helps to elucidate the story of the evolution and development of the inferior parietal lobule. On both sides the sulcus commences at a point slightly posterior to the temporal pole, having interposed between it and the pole a short anterior temporal sulcus (23*a*). Except for this separation from the anterior temporal sulcus, which Elliot Smith regards as a later development, the parallel sulcus is deep and unbroken right through to, and including, the upper and lower elements of the anterior occipital sulcus (26), which has already been described. The other associated branches of the parallel sulcus, namely the superior parallel (24)—the ascending I of Kappers—and the angular (25)—ascending II of Kappers—differ on the two sides. On the left side the superior parallel sulcus (24) is represented by two small disconnected sulci, which are little more than depressions, in the gyrus

supramarginalis; and increasing knowledge may show it to be found to be more than coincidence that the same arrangement occurs in the left hemisphere of the Bushwoman and in other brains of the Australian (1937, S. 10L and S. 4R).

In discussing the evolution of the parallel sulcus (1927, 1934) the point at which the anterior occipital sulcus is extruded from the parallel sulcus was referred to as the dynamic point of the inferior parietal lobule. It is marked × in Figs. 7 and 8. At this point the angular sulcus (25) may become joined with the parallel sulcus, or it may be separate as in both sides of this brain. On the right side the superior parallel sulcus is superficially confluent with the inferior postcentral sulcus (15*i*).

The angular sulcus (25), about which there is some confusion, is a new sulcus, which is sometimes found in the higher anthropoids, and is developed as a compensatory folding between the superior parallel sulcus and the upper part of the anterior occipital sulcus. It forms the boundary between the inferior parietal areas *a* and *b* of Elliot Smith (1907). On both sides of this brain it is represented in its normal relations; but in more highly developed forms, where increased expansion of the inferior parietal lobule takes place, an increasing number of compensatory foldings, often connected with the intraparietal sulcus, tends to obscure its identity (see Ingalls, 1914).

The extremely primitive nature of the inferior parietal region is further demonstrated on the left side by the simplicity of pattern of the intraparietal sulcus (16) which forms an uninterrupted arcade over the upper ends of the three branches of the parallel sulcus (24, 25, 26). There are no compensatory branches reaching down into the inferior parietal lobule except one small branch immediately in front of the anterior occipital sulcus (26) see Fig. 5. This extreme simplicity of pattern is absent in the brains of the Chinese and European which I have examined. At the risk of being tedious reference is again made to the brain of the Bushwoman because the pattern of the sulci of the inferior parietal lobule is practically identical with the pattern in this Australian child; the only exception lies in the fact that the angular sulcus is joined to the parallel sulcus. Reference is also made to the brain of the Negress depicted by Elliot Smith (1904, Fig. 25) and described by him as "exhibiting a greater assemblage of primitive features than probably any other human brain ever recorded".

The intraparietal sulcus (Figs. 1, 7 and 8)

The sulcus postcentralis (15) is a continuous sulcus parallel with the sulcus centralis (14), and on both sides it ends below in a right-angled branching lying across the lower end of the postcentral gyrus. This is the transverse postcentral sulcus (15*t*). The upper end of the superior postcentral sulcus presents an interesting condition when compared with the brains of the higher apes, in which the upper end of this sulcus most commonly forms a U-shaped branching embracing the upper end of the sulcus cinguli (10). Where this U-shaped sulcus is well defined there is no superior parietal sulcus; in other

words, there is no compensatory sulcus in the superior parietal lobule between the superior postcentral sulcus and the parieto-occipital arcade. As the expansion of the superior parietal lobule proceeds in harmony with that of the inferior parietal lobule and the upper half of the precuneus, the U-shaped sulcus becomes broken posteriorly to produce the superior parietal sulcus (37). In the brain of Marshall's Bushwoman a complete U-shaped sulcus is present on the left side and the detached posterior element on the right side. In brain 19 the original U-shape is indicated on both sides; but the superior parietal sulcus (37) has become detached and forms a simple compensatory sulcus in the superior parietal lobule.

The *sulcus intraparietalis pars horizontalis* (16*h*) is uncomplicated on both sides. On the left side it is joined to the postcentral sulcus anteriorly but is free posteriorly; and on the right side it is free anteriorly and is joined to the paroccipital sulcus posteriorly. The *paroccipital sulcus*[1] is very ill-formed, producing contraction of the parieto-occipital arcade. Posteriorly its horizontal part runs under the occipital operculum without a clearly defined transverse occipital sulcus (see dotted lines, Fig. 6).

The intraparietal arcades are simple in pattern. On the right side the supramarginal arcade arches over the ascending terminal limb of the fissure of Sylvius (Fig. 7), being formed by the sulcus postcentralis transversus (15*t*) anteriorly, the sulcus postcentralis inferior (15*i*) superiorly and the superior parallel sulcus (24) posteriorly. The superior parallel sulcus is separated from the postcentral sulcus by a buried gyrus. Next comes the angular arcade formed by the pars horizontalis which is broken, the outer limb of the sulcus parietalis transversus and in front the superior parallel sulcus. Within this arcade is the angular sulcus (25). The postparietal arcade arches over the bifurcated upper end of the anterior occipital sulcus (26) and is formed by the outer limb of the sulcus parietalis transversus, the sulcus paroccipitalis and the occipital operculum.

On the left side (Fig. 8) the arcades are not so clearly defined because the supramarginal and angular arcades have become confluent. The composite arcade is bounded by elements of the intraparietal sulcus and there are no compensatory branches passing downwards from the intraparietal sulcus into the inferior parietal lobule except the small sulcus lying between the composite arcade and the postparietal arcade. The whole arrangement clearly indicates the lack of expansion of the parietal region and is in keeping with the condition of the superior parietal lobule and the precuneus.

The frontal lobe

The precentral sulcus. On the left side this sulcus (5) (Fig. 8) appears as a continuous deep sulcus extending from a point close to the medial border of

[1] "The paroccipital sulcus consists of three pieces in an I-shape. Anteriorly the sulcus parietalis transversus—a transverse piece—posteriorly the sulcus occipitalis transversus—a transverse piece—and connecting the two a crosspiece" (see Shellshear, 1937, p. 356).

the hemisphere to the angle formed by the ascending anterior limb of the fissure of Sylvius and the main horizontal limb. Its various elements can be clearly distinguished from one another. The superior precentral sulcus (5*s*) (Fig. 1) is a triradiate sulcus with a short anteriorly directed limb and two posterior arms continuous with each other vertically. This triradiate form is the primitive simian form. The anterior limb constitutes the most posterior element of the superior frontal sulcus (11*a*). This pattern is so constant in lower forms that the anterior limb must be considered an integral part of the superior precentral sulcus.

The remainder of the precentral sulcus is composed of different elements which have become confluent. Above it is apparently confluent with the lower limb of the superior precentral sulcus, but a deep gyrus separates them. The sulcus then passes straight downwards to a point slightly below the inferior genu of the central sulcus where it gives off an anterior and a posterior branch. The anterior branch (5*a*) passes upwards and forwards into the middle frontal gyrus. It is the horizontal element of the inferior precentral sulcus of Cunningham (1892) and probably forms in part the homologue of the sulcus arcuatus of the apes. Below this the precentral sulcus is continued almost to the fissure of Sylvius, but the identity of the lower part is somewhat doubtful. It is, however, equally deep with the remaining part of the sulcus. Cunningham has pointed out that where the sulcus diagonalis is well developed the inferior precentral sulcus tends to fall short. It would appear then that the continuation of the inferior precentral sulcus in the left hemisphere is the sulcus diagonalis (13) and that the lower end of the inferior precentral sulcus is the posteriorly directed branch (5*b*). The inferior frontal sulcus (4) is confluent with the diagonal sulcus, a fairly common condition in the human brain.

On the right side the arrangement is somewhat similar. On the medial border there is a short sulcus praecentralis mesialis. This is separated from the superior precentral sulcus. This sulcus is triradiate; but the anterior limb has a different value to that on the left side and will be considered later. The superior precentral sulcus is separate from the inferior precentral sulcus which has the same form and relations as on the left side.

The superior frontal sulcus (11). I have previously discussed the morphology of the frontal sulci (1937); but because this brain reveals a pattern so highly suggestive of an intermediate stage between the more primitive extinct members of the human family and the higher races of man, it is profitable to discuss here the frontal sulci in general before dealing with the individual sulci in this brain. Before the work of Campbell, Elliot Smith, Brodmann and others on the microscopic and macroscopic structure of the brain, it was only natural that the mistake should have been made of confusing the inferior frontal sulcus with the sulcus rectus of the apes. Campbell's work (and thanks to the thoughtfulness of Mrs A. W. Campbell in giving his original papers to the University of Sydney I have had access to more than his published record) showed that the enlargement of the frontal region in man was produced by an

expansion of new cortex immediately anterior to the lower end of the central sulcus. Brodmann defines this development in his areas 44, 45 and 46 and shows, as do Campbell and Elliot Smith, that its superior boundary is associated with the inferior frontal sulcus. This sulcus therefore finds its highest expression in the higher types of man, although there are hints of its appearance in the brain of the higher apes. Kappers (1929, p. 3) clearly recognised the significance of the sulcus frontalis medius in the endocranial casts of fossil man, and called attention to the mistake in confusing it with the inferior frontal sulcus in the anthropoids. The condition of the middle frontal sulcus in the Australian (p. 394), taken in conjunction with the evidence of endocranial casts and the disposition of the cortical areas, seems to suggest that the difference between the anthropoid and human brain in this region is expressed in the greater compensatory folding along the line of the primitive sulcus rectus displacing this upwards and forwards. These foldings are expressed in the complex sulcus frontalis medius; so that whilst the homology cannot be exactly stated, it is probable that the sulcus rectus is represented in the human brain by the sulcus fronto-marginalis and the sulcus frontalis medius. It is only after the accentuation of the sulcus frontalis medius in the lower races that the further expansion of the frontal lobe finds its expression in the greatest length and depth of the sulcus frontalis inferior, and the more continuous folding of the sulcus frontalis superior. At the same time this further expansion causes a breaking up of the sulcus frontalis medius.

In the brain under discussion the earlier stage in the expansion of the frontal region is diagrammatically represented. On both sides the middle frontal sulcus (6, 7*a*, 7*b*) is the most prominent sulcus of the frontal lobes. On the left side it commences in the angle formed by the superior frontal sulcus and the superior precentral sulcus whence it passes as a continuous sulcus to the frontal keel where its anterior part constitutes the fronto-marginal sulcus of Wernicke (9). On the right side the posterior end of the sulcus frontalis medius is confluent with the superior precentral sulcus, and anteriorly with the element 7*a*. The element 7*b* and 9 are separate sulci.

The justification for regarding the posterior part of the sulcus as belonging to the sulcus frontalis medius is somewhat debatable. Eberstaller defined the sulcus frontalis medius as frequently commencing in a branching in the middle frontal gyrus. This branching has been numbered 6 in Kappers's figures (1929) and the position of this figure corresponds with the figures of this paper. Kappers (1929, Fig. 2, p. 185) depicts his interpretation of the frontal lobe in the endocranial cast of La Quina and there labels as sulcus frontalis medius the sulcus which corresponds in every way with that regarded as the sulcus frontalis medius here. This, together with the fact that the various components of the superior frontal sulcus (11) are clearly defined, makes it a reasonable assumption; moreover, the condition is in keeping with the Australian pattern generally. The form and relations of the sulcus fronto-marginalis (9) are typical on the two sides and call for no comment.

The *superior frontal sulcus* (11). On both sides this sulcus is broken into four pieces. This conforms to the Australian pattern in which the condition occurs in fifty per cent. of cases. It indicates a lowly degree of development and was noted by Cunningham who stated that: "In the Negro cerebrum this furrow appears to have a greater tendency to break up into separate pieces than in the European."

The *inferior frontal sulcus* (4). On the right side there are two sulci which appear to represent this sulcus. First, there is a short branch of the inferior precentral sulcus extending forwards towards the middle frontal sulcus (4?). It is more probable, however, that this is the anterior horizontal branch of the inferior precentral sulcus of Cunningham. Secondly there is the short sulcus (4) lying free in the inferior frontal gyrus which is the only representation of the inferior frontal sulcus. On the left side the sulcus frontalis inferior is more clearly defined and is confluent posteriorly with the sulcus diagonalis. Compared with higher types the sulcus is short. Anterior to the inferior frontal sulcus there is a diagonally placed sulcus representing the sulcus radiatus (3), and, perhaps, also the axial sulcus of the triangular operculum.

The simplicity of pattern of the frontal lobes is in harmony with the evidence for ill development in other areas of this brain; and in keeping with my previous description (1937, p. 404). The sulcus frontalis medius has a pattern which closely resembles that of Homo neanderthalensis as interpreted by Kappers. In the higher apes this sulcus is more often a continuous sulcus, while the superior frontal sulcus is fragmentary (see Mingazzini, 1928, Figs. 15, 24, 46, 49, 68, etc.). And, finally, the inferior frontal sulcus in the simplicity of its pattern displays the lack of development of the inferior frontal region.

The medial surfaces of the frontal and parietal lobes

In dealing with the medial surface of the occipital lobe it has been shown that the upper part of the precuneus is ill-developed on the left side and slightly better developed on the right side.

The medial surface of the frontal lobe is relatively narrow on both sides. On the left side the sulcus cinguli (10) is broken into three pieces, on the right side it is continuous. There is no sulcus paracingularis and there is an absence of radiating compensatory branches. I have elsewhere dealt with this region (1937, p. 375) and since this brain is so essentially similar to that description further details are unnecessary.

The tentorial surface (Fig. 11)

The lower end of the vertical retrocalcarine sulcus (19*v*) turns on to the tentorial surface at the occipital pole in both hemispheres; and is surrounded by a diminutive inferior polar sulcus (18*i*). On the left side two sulci are connected anteriorly with the inferior polar sulcus. The medial of these (36) lies

parallel with the retrocalcarine and anterior calcarine sulci and ends by turning inwards into the gyrus paradentatus immediately in front of the anterior end of the anterior calcarine sulcus. Just before its termination it enters into close association with the apex of the convexity of the sulcus collateralis (35). The striate cortex falls short of sulcus (36) so that this corresponds to Elliot Smith's sulcus paracollateralis—a sulcus limitans areae parastriatae. It accords with his description (1907, p. 201): "In the lingual gyrus there may be two sulci or only one; in the latter case the furrow may be a sulcus limitans areae striatae or a sulcus limitans areae parastriatae."

The sulcus connected with the inferior polar sulcus laterally (32) is a deeply operculated sulcus posteriorly, which passes along the tentorial border of the hemisphere almost as far as the anterior end of the temporal lobe. In its course it varies in depth and is broken by deep gyri. At the preoccipital notch it is confluent with the lower end of the anterior occipital sulcus. The posterior part of the sulcus extending as far forwards as the preoccipital notch is the inferior occipital sulcus (32); the middle part is the occipito-temporal sulcus (33) and the anterior part consisting of two branches is the inferior temporal sulcus (27).

The *collateral sulcus* (35) lies in the interval between the paracollateral and inferior occipital sulci. It commences in a transverse piece, sometimes named the transverse collateral sulcus, immediately in front of the inferior polar sulcus, and thence passes forwards on the tentorial surface to end within the inner branch of the occipito-temporal sulcus.

On the right side the pattern is slightly different from the left on account of a greater separation of the various sulci; but the above description of the left side is adequate for it.

The sulci on the orbital surface of the frontal lobe show nothing of moment and are seen in Fig. 11.

GENERAL SUMMARY

The description of the brain of a girl, aged 12, said to be that of an aboriginal Australian, has been given.

The brain reveals a grouping of primitive features, evidence of ill-filling and a lack of development in the higher association areas. The conditions found are in harmony with those found in the Australian brain generally and are very similar to those found in the brain of Marshall's Bushwoman.

In the occipital region a deep operculum occipitale is present on both sides in association with vertical folding of the area striata. The transverse occipital sulcus cannot be defined. On the medial surface the form of the parieto-occipital fissure is in harmony with an ill-development of the precuneus and superior parietal lobule.

In the parietal region the form of the parallel sulcus and its associated branches, together with the form of the intraparietal sulcus, presents a picture in keeping with the primitive pattern of the sulci related to the visual areas and

demonstrates that the pattern of the sulci can be used to indicate the degree of development of the parietal region.

In the frontal region the broken condition of the superior frontal sulcus, the well formed middle frontal sulcus and the diminutive inferior frontal sulcus are directly comparable with Kappers's description of the endocranial cast of Homo neanderthalensis; this reveals a lack of frontal development commensurate with the lack of parietal development.

Throughout the description frequent references are made to my previous findings on the brain of the aboriginal Australian. The conditions in this brain strikingly confirm that account and this description forms an adequate summary of the brain of the aboriginal Australian.

LIST OF REFERENCE NUMBERS IN THE FIGURES

1 S. subfrontalis
2 Fissure of Sylvius
2*a* Anterior ascending limb of the fissure of Sylvius
2*h* Anterior horizontal limb of the fissure of Sylvius
3 S. radiatus
4 S. frontalis inferior
5*a* Anterior horizontal branch of the s. praecentralis inferior
5*b* Posterior branch of the s. praecentralis inferior
5*i* S. praecentralis inferior
5*s* S. praecentralis superior
6 Posterior branching of s. frontalis medius
7 S. frontalis medius
7*a* Posterior part of s. frontalis medius
7*b* Anterior part of s. frontalis medius
9 S. fronto-marginalis
10 S. cinguli
11 S. frontalis superior
13 S. diagonalis
14 S. centralis
15*i* S. postcentralis inferior
15*s* S. postcentralis superior
15*t* S. postcentralis transversus
16 S. intraparietalis
16*h* S. intraparietalis pars horizontalis
16*pt* S. parietalis transversus
17 S. occipitalis transversus
18 Operculum occipitalis
18*i* S. polaris inferior
18*p* S. praelunatus
19*v* S. retrocalcarinus verticalis
20 S. retrocalcarinus
20*a* S. calcarinus anterior
21 F. parieto-occipitalis
21*i* Incisura parieto-occipitalis
21*l* S. limitans praecunei
21*p* S. paracalcarinus
23 S. parallelus
23*a* S. temporalis anterior
24 S. parallelus superior

25 S. angularis
26 S. occipitalis anterior
27 S. temporalis inferior
30 S. subparietalis
31 S. praecunei
32 S. occipitalis inferior
35 S. collateralis
36 S. paracollateralis
37 S. parietalis superior
O.S. Bottom of occipital opercular sulcus

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REVIEWS

The Hypothalamus. By W. E. Le Gros Clark, John Beattie, George Riddoch, Norman M. Dott. (Edinburgh: Oliver and Boyd.) 1938. Pp. xii + 211. Figs. 23 + 42*b*. Price 12*s*. 6*d*.

This book arises out of the lectures given in the Edinburgh University under the benefaction known as the Henderson Trust. The necessary editorial work has been performed by Prof. Brash.

The four authors, Prof. W. E. Le Gros Clark, Prof. J. Beattie, Dr Riddoch and Dr Dott have published the lectures they gave on the anatomy, the physiology, the clinical aspects, and the surgery of the hypothalamus. The interest in the clinical and surgical aspects of the hypothalamus has now become very great and the subject has been immensely developed by the experiments and investigations of anatomists and physiologists. Prof. Le Gros Clark and Prof. Beattie occupy a very distinguished place amongst these.

The hypothalamus may be regarded as a set piece in neurology, the object being to fit its nuclei and fibre tracts with their proper functions. The methods evolved permit of the stimulation or destruction of these, and the results are reviewed in terms of centres, connecting tracts, and functional change or deficiency. The inability at this moment to reconcile the conflicting anatomical results has led to some retreat from the logical implications of the problem and the methods used. Prof. Beattie proposes a twofold division of the hypothalamus—an anterior vagal or parasympathetic, and a posterior sympathetic area. Dr Riddoch insists that diffuseness is always a characteristic of sympathetic activity.

It is natural that each author should approach his task from the direction in which his own researches have led him.

Prof. Le Gros Clark gives an excellent account of the anatomy. The difficulties of what he refers to as "terminological differentiation" are abolished for his readers by an easily comprehensible account of the nuclear pattern in the mammalian and a special account of the anatomy of the human hypothalamus, including the effect upon the latter of the angulation of the basis cranii. Prof. W. Le Gros Clark faces consistently the issues of accurate identification of nuclear masses by reasonable criteria, of the accurate determination of fibre tracts by experimental methods, and of giving due weight to evolutionary trends. Obviously there must be a shift imposed on a visceral correlation region when there is a change over from a brain under the domination of olfaction to one with extensive neocortical activities. The anatomical and physiological effects of such a change are as yet only apprehended in general terms. Again, anatomy suggests a great extension of the nucleus paraventricularis whose cells have peculiarities that may be interpreted as secretory. The anatomical and chemical relations of the hypothalamus are not yet completely understood, but enough has been done to suggest that anatomy does not easily support such conceptions as that the hypothalamus works as a whole, that all its parts are equivalent, or at best some sort of duality can be seen in its cranial and caudal portions. For instance, the mammillary region is anatomically the most differentiated part of the hypothalamus, yet it is implied that its functions are the most diffuse.

Prof. Beattie approached his task, as he says, under the inspiration of Barcroft's book on the theme that the constancy of the internal environment is the condition of a free life. The phrase "free life" has always been somewhat mysterious. The most likely meaning is that animal activity is least affected by external changes when the internal state can be kept constant. For the present purpose a free life is interpreted as being secured by activity of the central nervous system. Prof. Beattie is concerned therefore to review all the vegetative activities of the body and show that these are wholly or in part related to the hypothalamus. The adoption of this plan has diminished the consideration given to the immediate problem of relating minute

structure and function, but has given us an extremely interesting analysis of a whole series of complex bodily reactions.

It is natural for anatomists to be concerned with the achievements of their colleagues. It would, however, be ungracious not to commend highly the descriptions and analyses of hypothalamic syndromes which Dr Riddoch has portrayed. The surgical approach to the hypothalamus presents the greatest difficulty. The manner in which this has been overcome enables Dr Dott to give an account of surgical achievements that are altogether remarkable.

It is a great pleasure to welcome this scientific and practical contribution by anatomists and clinicians to medical science.

Studies on the Physiology of the Eye, Still Reaction, Sleep, Dreams, Hibernation, Repression, Hypnosis, Narcosis, Coma, and Allied Conditions. Reissue with Supplement and new Index. By J. GRANDSON BYRNE, M.D. xii+424 pp. 48 illustrations. Royal 8vo. (London: H. K. Lewis & Co.) Price 40*s.* net.

The book is made up of a number of scientific papers. They were intended for publication in scientific journals, but the author found no time to devote to preparation of the material for publication. The opening chapter describes the paradoxical pupillary reactions following lesions of the afferent nervous paths, but there is no mention of the normal reactions. Later chapters deal with similar phenomena in accommodation ("Widening and narrowing of the lens"). The book ends with chapters on normal pupillary reactions and the mechanism of accommodation. In the middle of the book there are a few chapters on sleep, dreams, hibernation, repression, hypnosis, etc. The text appears to be identical with that published in 1932 except for the addition of a supplement of 15 pages on the effect of stimulation of the cerebral cortex on movements of the iris and membrana tympani. There is also a new index. Most of the references are pre-war. Although the book is obviously not designed to help beginners to understand the actions of the sympathetic and parasympathetic nervous systems on the intrinsic and extrinsic muscles of the eye, the advanced worker may find something of interest since the author has performed a large number of experiments under a variety of conditions and the protocols are full. The actions of drugs such as atropine, adrenaline, pilocarpine, etc. on the ocular muscles are fully investigated but unfortunately these actions are not discussed in the light of modern knowledge on the sympathetic and parasympathetic nervous systems.

A History of Women in Medicine. By KATE CAMPBELL HURD-MEAD, M.D. xvi+569 pp. Illustrated. (Haddam, Conn.: The Haddam Press.) 1938. Price 6 dollars.

It will come as a surprise to many who read this book to find that the battle for entry to the medical profession waged by Elizabeth Garrett Anderson and many others during the sixties and seventies of last century was in reality a struggle for the re-opening of the registers to women.

Dr Hurd-Mead has done a great service in giving a chronological account of the doings of women in medicine from the earliest times almost to the present day. The story of the achievements of medical women still living she is to give in a second volume.

This record shows clearly that their great value as practitioners in one age, and their poor performance in another, depends directly upon their educational facilities and social freedom at the time in question.

In Tudor times, for example, women were given a liberal education—classics, mathematics, modern languages and music—by the same tutors who instructed their brothers; they also received training in cooking and needle-work.

Research has shown that in this period there was very little restriction of the practice of medicine by women. When the College of Physicians was created by the Act of 1511 provision was made for the training of both men and women—the course lasted eight years in some cases—and candidates of both sexes were tested by the same examinations in order to prove their qualifications for practice (pp. 521–3).

The Act of 1511 was ratified in 1518 and again in 1523: it was never repealed. What happened was that it was superseded in 1858 by an Act which ignored the existence of women as students of medicine and as practitioners unless they were graduates of some recognized foreign university (for women had never been admitted to English universities).

That the quality of women's work in medicine had sunk low in the early years of the nineteenth century there is no doubt, but the same is true of their general education. The rapid increase in wealth of the middle classes at this time coincided with the withdrawal of their women folk "whom they could afford to keep", from the world at large and confined them to the world of another's house and garden. Thus it came about that the women who moved freely in the world and knew its urgent medical needs were for the most part very poor and unlearned and little fitted to present themselves for examination by the College of Physicians on the same terms as the young men from the universities.

So the women practitioners of medicine in this country came to be represented by a steadily deteriorating group of midwives to be finally personified in Sairey Gamp.

But this is a mere episode in the long and honourable history of women in medicine. In 1876 when increasing knowledge of social conditions had aroused middle class women from their lethargy to seek again co-operation in the medical work of the nation the registers were re-opened.

The bibliography alone should ensure a place for this book in all medical libraries.

Elementary Anatomy and Physiology. 4th Edition by MARY REES MULLINER, M.D. (London: Henry Kimpton.) 1938. Pp. 542, 312 figs. Price 21*s.* net.

This book presents a satisfactory selection of the facts of anatomy and physiology, and is suitable for students of hygiene, physical training, massage and pre-medical work generally.

Die Periphere Innervation. 7th Edition by EMIL VILLIGER. (Leipzig: Wilhelm Engelman.) 1938. Pp. viii+177, 71 text-figures. Price R.M. 9.

The purpose of this book is to present briefly the origin, course and distribution of the spinal and cranial nerves, including also a brief account of the autonomic nervous system and allusion to some of the more important pathological conditions.

Kolloidchemie des Protoplasmas. 2te Auflage von W. LEPESCHKIN. Wissenschaftliche Forschungsberichte, Naturwissenschaftliche Reihe, Bd. 47. (Dresden and Leipzig: Th. Steinkopf.) 1938. Pp. xii+244, plate I, 7 text-figures. Price R.M. 18.

This book contains an extensive review of the literature upon the physics and chemistry of animal and plant protoplasm. After a short introduction to colloidal chemistry the author proceeds to discuss the physical state of cytoplasm, karyoplasm, and cell inclusions such as granules, chromatophores, chondriosomes, fibrils, vacuoles, etc. In a further section the chemistry of these structures is detailed, considerable attention being paid to the alterations they undergo when exposed to temperature changes, mechanical trauma, electrical currents, radiant energy, changes in concentration of electrolytes and narcotics. Chapters are also devoted to the chemical and physical changes cells undergo during mitosis, fertilization, ageing and death.

The Anatomy of the Domestic Animals. By SEPTIMUS SISSON, S.B., V.S., D.V.Sc. Revised by JAMES DANIELS GROSSMAN, G.Ph., D.V.M. 3rd edition. 972 pp. 737 figs. London and Philadelphia: W. B. Saunders Company, Ltd.

This well-known text book of the anatomy of the domestic animals for students of veterinary medicine is now in its third edition, but Professor S. Sisson, of Ohio University, its original author, has been succeeded by Professor D. Grossman.

The book sets forth the descriptive macroscopic anatomy of each bodily system of the horse, ox, sheep, pig and dog in turn. A brief but adequate account of the systematic anatomy of the chicken is added to the present edition. The text is restricted to the descriptive features that can be elucidated by the ordinary method of dissection. The book contains about 950 pages of text, 770 illustrations—the blood vessels are coloured—and a sufficiently detailed index.

For its purpose the book is admirable.

IN MEMORIAM

THOMAS WINGATE TODD (1885–1938)

In the closing days of 1938 came the sad news from America of the death of T. Wingate Todd, Professor of Anatomy in the School of Medicine, Western Reserve University, Cleveland, Ohio, at the age of 53. He was an Englishman, born in Sheffield in 1885, the son of a dissenting clergyman, of Scottish extraction or of Scottish birth. His education and his progress in life depended on his own efforts; he was born with an insatiable desire for work, and with an enthusiasm for it which grew as his years and burdens accumulated. Entering the University of Manchester, he took the degrees of M.B., B.Ch., and thereafter held resident posts in the Infirmary, Manchester. Probably his first bent was towards surgery, for he became a Fellow of the Royal College of Surgeons, England, in 1911, at the age of 26.

After finishing his posts in the Infirmary he returned to the department of Anatomy of his University, then under the direction of Prof. A. Young. As Prof. Young's health was then failing, much of the teaching in the department fell on Wingate Todd, until 1909 when the late Sir Grafton Elliot Smith was appointed to the chair in Manchester. Wingate Todd served under the new professor until 1912 when he was invited to fill the chair of Anatomy in Cleveland, Ohio, which he held until the date of his death—a period of 26 years. The late Prof. J. J. R. Macleod, another British subject, had been appointed to the chair of Physiology, in the same University, some time before the arrival of the new Professor of Anatomy.

There was no dearth of young anatomists in America when Wingate Todd was appointed to Cleveland; Franklin Mall had bred a large and promising school, equipped with the latest technical methods; but the opinion then held by many medical men in the United States was that the teaching of English anatomists stood nearer to traditions of practical medicine than did the more scientific methods that had their origin in Baltimore. However ill-founded this opinion may have been, there is no doubt that the great school of Anatomy which Wingate Todd established in Cleveland was English in its method and in its outlook. At Manchester he had become interested in two problems; one was the origin and growth of bone; the other was the nerve-lesions which attend certain cases of cervical rib and which may lead to changes in arterial structure. His first communication on the histology of growing bone appeared in this *Journal* in 1913 (vol. XLVII, p. 177). His last—on the growth of bone—took the form of an atlas—*Atlas of Skeletal Maturation* (St Louis, 1937). This contains 75 plates—each showing a certain stage in the ossification of the bones of the hand—as revealed by X-ray examination. His aim was to provide standards of the normal process of ossification

Geoffrey Landesman

THOMAS WINGATE TODD

UNIV. OF MICH.

for other workers. He had planned subsequent volumes which would cover the whole human skeleton from birth to maturity. Between his first paper in this *Journal* and the publication of the *Atlas*, there appeared from his pen, and from the pens of his host of pupils and collaborators, a continuous succession of papers on skeletal growth. Many of these appeared in the *American Journal of Physical Anthropology*, others in the *American Journal of Anatomy*, in the *Anatomical Record* and not a few in this *Journal*. He desired to have the foundations of our knowledge of the growth of the human body laid on a broad and sure foundation. He was a foundation layer.

His first paper on the pressure effects produced by cervical ribs on the nerve supply of the upper limb—particularly on its autonomic supply—appeared in the *Lancet* of 1913 (vol. I, p. 1371), and his final observations were summed up in the *Annals of Surgery* (1922, vol. LXXV, p. 105). He regarded his enquiry into the responses of the alimentary tract, which he began on his students in 1927, as a continuation of his observations on the function of the autonomic system (*Ann. Int. Med.* 1927, vol. I, p. 420). In reality it was a search into the motility of the alimentary canal with a view of ascertaining the range of response and of rate met with in health—and under varying mental states. He summed up his observations on the alimentary tract in his Beaumont Lectures published in book form under the title *Behaviour Patterns of the Alimentary Tract* (Baltimore, 1930).

His most ambitious undertaking—the greatest of its kind ever accomplished by an individual effort—was begun soon after his arrival in Cleveland. His enthusiasm and boundless energy drew to him men of means and of goodwill. The Hamann Museum was established in connexion with his department. There was nowhere in the world an extensive collection of human skeletons—of which the age, sex and race had been verified at death. Without such a collection all inferences relating to age changes, the closure of sutures, the fusion of epiphyses, the degree and nature of sexual differentiation, the range in characters which were supposed to mark one race off from another, were in reality of the nature of guesses. He set about building up a documented collection of skeletons; in 1933 he had at his disposal, and at that of the many workers who joined him, over 2400 human skeletons—juveniles and adults—with all the needed data attached. He also assembled large collections of anthropoid skeletons and of other mammals—especially those which revealed the processes of bone growth and epiphyseal union. From 1923 onwards there appeared a rapid succession of papers founded on data provided by his skeletal material in the Hamann Museum. A list of these publications is beyond the scope of this notice. They appeared in the *American Journal of Physical Anthropology*, in the *Journal of Anatomy*, the *American Journal of Anatomy* and in the *Anatomical Record*. His aim was to lay a foundation on which others might raise a physical anthropology of a truly scientific kind. His observations on the changes undergone by the skeleton from maturity to old age opened up a new field of knowledge. He was joined by Dr W. M.

Krogman who did for anthropoid apes what the Master and his pupils had done for the human body. His latest publications on this subject appeared in the more recent volumes of the *American Journal of Anatomy*—where he analysed the effect of thyroid deficiency on cranial and facial growth, and contrasted the order of epiphysial fusion in various orders of mammals.

In 1926 he entered a yet more strenuous field of observation. He aimed at laying our knowledge of the growth of children on a more intimate and exact basis than had been possible before. Most observers had confined their attention to two aspects of the growth of the body—its increase in stature and in weight. He aimed at an exact X-ray record of the growth changes in the bones—from birth to maturity accompanied by full clinical notes of each individual. He had to leave his department and go into the city of Cleveland to obtain the information he was in need of. This entailed a health crusade—an appeal to those that would listen to him—an appeal for opportunity and for financial aid. The Brush Foundation came to his aid. By 1930 he had under systematic examination 800 children—born of healthy and well-to-do Clevelanders. Each child had to be examined four times a year; exact records had to be kept of each examination. The organization of such an undertaking was occupation enough for any one man; but he kept all sides of his department going at the same time; his head and hands were as fully occupied as those of the President of the country in which he worked and prospered. In this enquiry, too, publication followed publication with amazing rapidity; he was never too tired to spread his knowledge by public lectures and by communications to medical societies. When the White House Conference met in 1932, it adopted his standards for measuring health and growth. His contributions to the publications of the Conference contain many new and valuable observations on the anatomy and growth of children. The *Atlas* which he completed represents the first sheaf of the harvest he proposed to reap from his Children's Clinic. Those who desire to become acquainted with this part of his work will find references to the numerous publications issued from his Children's Clinic in the list of literature published in the *Atlas*.

One important side-issue of this enquiry related to the growth of the face. He opened a field of investigation—which in the hands of others has reached vast dimensions. He perceived that if orthodontic treatment was to become effective, it must be based on a knowledge of the normal and disordered growth of the face in childhood.

Since the time of John Hunter there has been no one with such an unquenchable desire to accumulate material and to build new foundations for the biology of the human body. No one has worked his own body and brain so near to breaking point as he did—year after year, regardless of health and comfort. Perhaps he attempted too much; his writing and thinking often bear the mark of haste and of breathlessness; his thoughts were often left with ragged edges; but he has left a great heritage, if only a man fit to shoulder his burden and reap his harvest can be found to succeed him.

He was very happy in his marriage; Eleanor Pearson whom he married before taking up his appointment in Cleveland has been a splendid help-mate to him, as well as rendering great service to the city of her adoption. "Adoption" is, perhaps, not the right word, for Mrs Wingate Todd, like her husband, never renounced allegiance to the land of her birth. But when she and her husband spoke of "home"—their three children, all rising to take a high place in life, reminded their parents that their "home" was America. In the Great War Wingate Todd obtained a commission in the Canadian Army Medical Service and came to Europe with troops.

In connexion with his nationality I may quote from a letter which reached me only two months ago. A number of British anatomists, at the instigation of Sir Sidney Harmer, wished to see him a Fellow of the Royal Society, and prepared a certificate of nomination—now alas! useless. In this letter he wrote: "I had put the 'Royal' out of my mind because a British subject resident abroad must not expect his country to take much heed of him. I had also put the National Academy out of mind because not being a naturalized American citizen I could not expect the country of my adoption to pay much heed. I shall of course welcome your generous efforts but shall not be cast down if nothing happens." Our efforts were too late.

The photograph reproduced was forwarded in the last envelope addressed by him to me. It reached me three days after the news of his death.

ARTHUR KEITH.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

November 1938

RECORDED BY

A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

NOVEMBER 1938

The Annual Meeting of the Society was held at St Bartholomew's Hospital Medical College at 3 p.m. on Friday, 25 November 1938, with the President (Professor ALEXANDER LOW) in the Chair.

Dr G. SIMON read a paper on "Tomography in the teaching of radiological anatomy".

The general principle of the method consists in moving the X-ray tube and X-ray film in such a way relative to each other, that a certain layer in the subject is clearly defined, while the rest of the image is blurred out by the movement. By varying the relative amount of movement clear images can be obtained through different layers of the subject.

In the simplest form of tomogram devised by Twining, the tube moves parallel to the film. It is often possible to arrange for this on existing apparatus by some very simple system of connecting rods, such as that demonstrated.

Radiograms of the human thorax are shown demonstrating a layer situated posteriorly, with the clavicles and anterior parts of the ribs invisible, and a layer anteriorly, with the clavicles and sternum showing, but no shadow of the scapula or posterior parts of the ribs.

Professor WOOLLARD was informed that the delay in the adoption of routine tomography in this country was purely economic: British radiologists had now succeeded in substituting an equally efficient but considerably cheaper apparatus for that used and exported from abroad, and this new apparatus was in process of general adoption.

Dr CAVE commented upon the accuracy of tomographic findings from his experience of their post-mortem confirmation.

Dr A. M. CARLETON read a "Note on the effect of colchicine on the skin of young rats".

The rate of mitotic division in the skin and hair follicles of eight young rats was studied by the colchicine technique. Twelve different surface areas were examined. With the possible exceptions of the regions above the umbilicus and in front of the knee, which showed increased rates, the average rate of growth does not vary very much over the surface of the body, having an average of 1·16 % with extremes of from 0·8 to 1·5. There appears to be a steady diminution in the rate of cell division from the first day onwards, from 1·8 % on the first day to 1·1 % at 6 weeks.

Colchicine is very uncertain in its effects, causing no rise in mitotic rate in some cases, a local but not a general rise in others, and in one out of the eight rats an average rise of 15 % with extremes of 2·7 and 22·4.

Mr RICHARDSON welcomed these findings in view of the general but uncritical acceptance of colchicine as an indicator of cellular growth rates. Whilst colchicine did indeed act upon dividing cells (affecting particularly the meta-

phase of the mitotic spindle) it appeared further to have the property of activating cell division itself: following its use, the final histological picture was highly complex and difficult of accurate analysis.

Professor WOOLLARD suggested that repeated biopsy might be a useful method of determination of cellular activity in such experiments.

Professor BEATTIE was informed that the same uniform sample of colchicine was employed throughout the investigation.

Dr BAXTER commented upon the regional waves of activity recognizable in the skin of the individual animal and upon the consequent difficulties inherent in the correlation of experimental findings in different animals.

Professor W. J. HAMILTON and Mr J. H. GOULD read a paper on "The normal oestrous cycle of the ferret".

The vaginal smear of the ferret has been investigated and correlated with the changes occurring in the genital tract during the oestrous cycle. The vulval size has been found to be not wholly reliable as an indicator of the state of the animal, especially in the early stages before full heat. The smear shows the typical rodent stages at anoestrus and full oestrus, but, owing to the much longer time taken by the ferret to come into full oestrus, there are subphases in the pro-oestrum which have not been recorded before. These subphases are:

(i) Period of polymorphism and polychromasia.
(ii) Period of acidophil polygonal cells with distinct nuclei.
(iii) Period of acidophil squamous cells with pycnotic nuclei.

In metoestrus the smear reverts slowly to the anoestrous state with the presence of syncytia. In pregnancy a similar reversion occurs but shows no syncytia. No diagnostic phase characteristic of pregnancy has been found.

The vagina shows a cyclical cornification which commences in the upper vagina at Stage II of the pro-estrum and reaches a maximum at oestrus. This cornification then disappears in metoestrus and pregnancy.

The fused horns and cervical region are sites of persistent leucocytosis. The epithelium shows a cyclic proliferation which is synchronous with that of the vagina. The cell type varies from two-cells thick (cubical cells) to two to seven cells thick (squamous cells). The horn undergoes the changes normally associated with oestrus. The ovaries show an increasing amount of vascularity as oestrus approaches and the number of young ova are at a maximum at anoestrus and full oestrus. Follicles of full pre-inseminal growth were found in the ovaries only when the smear showed typical oestrus.

Colchicine investigations revealed the onset of growth throughout the tract Stage I of the pro-oestrum—a maximum was attained by Stage II and remained at this stage until full oestrus. In the ovaries it was noticed that growth was most apparent in the follicles from the stage possessing five to seven layers of follicle cells until a small cavity had been formed. Glycogen showed an increase as oestrus approached. The amount was only slight except in the horns. Trypan blue was found in the reticulo-endothelial system and the deposition in the tract showed an increase reaching a maximum at oestrus.

Dr ZUCKERMAN enquired as to the authors' conception of oestrus and their criteria for the determination of full oestrus: he seriously questioned the validity of vulval swelling and vaginal smear cytology as reliable indices of

a phase of behavioural activity. Professor HAMILTON replied that in his experience a correlation did exist between vaginal smear histology and the successive phases of the oestrous cycle: thus a smear indicative of mid-oestrus was invariably associated with complete absence of ripe ovarian follicles, whilst maximal vulval swelling was accompanied by maximal occurrence of such follicles.

Professor NICOL remarked that the authors' present findings in connexion with vital staining and glycogen distribution largely confirmed his own results in ferret material, despite some differences in time-incidence and in the topography of vitally stained endometrial cells. He disagreed with the view which regarded these cells as scavengers, and suggested for them instead a histiotrophic function. He had observed in experimental ferrets a considerable intolerance to trypan blue: he asked what daily weight variations the authors' animals had manifested during the period of trypan blue injection and further, whether the endometrium of these animals had manifested certain large cells containing brownish granules.

Professor HAMILTON replied that certain of his injected ferrets had died; others, however, had tolerated trypan blue extremely well. He had not observed the peculiar large cells with brown staining granules referred to. Glycogen in the genital tract appeared to increase in quantity as the cycle moved from anoestrus to full oestrus.

Messrs W. J. HAMILTON, E. C. AMOROSO and W. B. GRIFFITHS read a paper on "The first finding of the tubal ovum in the goat".

Two goats were employed; one furnished a two-celled ovum; from the other two eggs were recovered, each with seven or eight cells. Photomicrographs of the specimens were made in Locke's solution while they were still alive and fresh. As seen from the photographs, the goat egg is typical of other mammalian eggs.

Professor J. P. HILL commented upon the interpretation as polar bodies of the small masses found lying in the middle of the segmenting egg—a curious site for true polar bodies, but Professor HAMILTON confirmed from other material the accuracy of this interpretation. Professor NICOL also discussed this paper recalling the excellent pioneer work of MacLaren in the same field.

Dr E. C. AMOROSO and Mr W. B. GRIFFITHS read a paper on "The living early developmental stages of the domestic mammals".

Fresh and living ova of the dog, cat, cow, sheep and goat were studied in various stages of development. Their features are described. Detailed measurements of eggs of various stages are given. The rates of progress of eggs through the genital tract are compared in the several species studied.

Drs M. L. GILCHRIST and L. H. DUDLEY BUXTON read a paper on "The relation of finger nail growth to nutritional status".

The nail growth was examined of a number of children from elementary schools in Leytonstone, East London, who had been independently graded into three groups, A, B and C, according to their nutritional status. Considerable individual variation in nail growth was found, but examination of the group means showed that while there was no difference in the rate of nail

growth between groups A and B, the nails of poorly nourished children in group C grew more slowly. The evidence suggests that the nail growth of well-nourished children shows less variation than that of the more poorly nourished.

Replying to Dr WEDDELL's query as to the method adopted of assessing nutritional status Dr GILCHRIST explained how a clinical standard of such status was established, which though difficult of adequate description proved satisfactory in practice.

Dr G. WEDDELL read a paper on "The frequency of double epiphyses in the metacarpals and metatarsals of Man".

Of the suggestions which have been made in regard to the reversed arrangement of the epiphyses in the first and other metacarpals and metatarsals, the most probable theory is that all these bones were originally ossified like long bones with a diaphysial and two epiphysial centres. The evidence for this suggestion consists of the following:

(*a*) Anatomical dissection. This has revealed an occasional additional epiphysis for both first and second metacarpals and first metatarsal (Thompson 1869).

(*b*) Blood supply. The angle of entrance of the nutrient artery into the shaft of the metacarpals varies and is in some cases the reverse of that which usually occurs (Anseroff, 1936).

(*c*) Radiographs have been published showing metacarpal bones all having two epiphysial centres (Monteiro & Carvallio, 1938).

Radiographs of two hundred sets of metacarpal and metatarsal bones of healthy school children between the ages of 4–8 years have been examined. It was found that no radiograph could be said to conform exactly to the classical pattern. Definite double epiphyses and well-marked pseudo-epiphyses were seen in 80 % of cases for the first metacarpal, 50 % for the second, 6 % for the third and fourth, and 48 % for the fifth. In the foot a true or well-marked pseudo-epiphysis was seen on the distal end of the first metatarsal in 67 % of cases. No other metatarsals showed indications of double epiphysial formation. In the hand careful examination showed that in many cases the bone was nicked on one side only at its proximal or distal end, but that the trabecular conformation in conjunction with the incision clearly indicated that an attempt at epiphysis formation had occurred. These were grouped together separately and showed fairly even distribution between the metacarpal bones. The first metacarpal 18 %, second 32 %, third 22 %, fourth 30 %, fifth 32 %. No significant sex variation was discovered in any of these X-rays, and with one exception all differences from the usual pattern were bilateral.

A statistical analysis of radiographs of children between the ages of 4–8 years is in accordance with the theory that metacarpals and metatarsals were originally developed in a similar manner to long bones. It also indicates that the most common position for marked double epiphysial formation is in the first, second and fifth metacarpals, but only in the first metatarsal.

Professor NICHOLSON demonstrated a slide showing synoptically the various recorded patterns of adventitious epiphyseal formations in the bones of the thumb. Dr BRAILSFORD submitted (by proxy) his personal findings concerning the occurrence of extra epiphyses in a series of some 200 children, from 1 to 2 years old: his comments were supported by selected radiograms.

Dr HAINES urged the need for a clear distinction between epiphyses and pseudo-epiphyses, detailing the histological differences observable between these two mechanisms of bone growth. He regarded a pseudo-epiphysis as a region wherein length increment of a bone is absent and raised the question as to whether pseudo-epiphyses represented a phylogenetic degradation of true epiphyses.

Dr DE BEER stressed the danger of equating the phylogenetic with the histogenetic picture, since ontogeny did not recapitulate phylogenetic phases. Professors WOOLLARD and DURWARD also discussed this paper, and Dr WEDDELL replied.

Professor H. H. WOOLLARD and Mr A. HARPMAN read a paper on "Observations on the coelenterate nervous system".

The forms studied were *Actinia equina*, *Cyanaea capillata*, and *Chrysaora isosides*. The stain impregnations used were methylene blue and gold chloride. Nerve cells and fibres were found most abundantly in the subumbrellar region adjacent to the margins where the so-called sense organs are found, and in the tentacles.

The nerve cells are usually bipolar, a nerve fibre process arising from each extremity of the cell. Occasionally a multipolar cell is found, but these are infrequent. The cells contain granules (stainable with methylene blue) which may extend for a short distance into each process. There is, however, no distinction between the processes; there is no differentiation into dendrite or axon; and neither medulla nor neurilemma is present.

The fibres are usually varicose and have minute lateral spikes all along their course. They may be thick or thin. This distinction depends on the distance the fibre has travelled from the parent cell. At the cell all the fibres have the same thickness.

The fibres are so arranged that larger concentrations of nerve material can be found in certain parts but within each group the fibres run in every direction. They may run parallel, cross each other obliquely or transversely or commonly one fibre will twist itself round and round another. This entwining of fibres is a very characteristic relation. The fibres pass by the cells having perhaps contact but forming no pericellular investment. Once an ending resembling a "bouton" was seen. Fusion does not occur, and no true nerve net is formed.

Pericellular endings on muscle cells occur and they seem in every way comparable to the endings found on unstriped muscle in the mammal. Processes coming from neuro-sensory cells were seen, but they could only be traced for a short distance. Presumably they enter the plexus of nerve fibres, but the details were not observed.

These findings are in accord with those of Schäfer (1878) and Bozler (1927). They are not in accord with those of Apathy, Bethe and others whose descriptions have led to the general acceptance of the notion that the coelenterate nervous system is a nerve net.

Professor WOOLLARD emphasized the structural discontinuity of the invertebrate nervous system, and the entire absence of evidence as to any generalized neural reticulum: present findings confirmed the pioneer researches of Schäfer made half a century previously. Professor BOYD welcomed this paper as a timely corrective of much erroneous continental teaching on the subject.

Professor DAVIES was informed that polarization in the coelenterate nervous system occurred physiologically. Dr WEDDELL recounted his experience of the technical difficulties attendant upon staining of the invertebrate neural mechanism.

The following paper by Dr WHEELER HAINES on "The epiphyses of *Sphenodon*" was taken as read.

The epiphyseal region of *Sphenodon* consists at first of a relatively large mass of hyaline cartilage with a well differentiated growth cartilage lying convexly over the end of the shaft. Later a large secondary centre of calcification is formed, and this persists for a relatively prolonged period. The calcified centre is then replaced by bone and marrow, probably derived directly from the neighbouring perichondrium. Finally, the growth cartilage is destroyed, and the bone of the epiphysis and of the shaft becomes continuous. As compared with primitive reptiles, the secondary centres of calcification and ossification, and the destruction of the growth cartilage, are specializations, while, as compared with lizards, the long persistence of the calcified centre is a relatively primitive feature.

The endochondral bone is arranged in longitudinal trabeculae, but these are directed towards the growth cartilage, not towards the articular surface as in primitive reptiles, and the structure is more refined. The arrangement of the epiphysis and endochondral bone is consistent with the remote common origin of *Sphenodon* and the lizards.

DEMONSTRATIONS

At this Meeting of the Society the following Demonstrations were given:

(1) E. C. AMOROSO and W. B. GRIFFITHS: Specimens illustrating their communications.

(2) A. J. E. CAVE: (*a*) Seventeenth century ivory anatomical mannikins.

(*b*) Neolithic and modern scapulae showing an adventitious epiphysis on the dorsal aspect of the spine.

(*c*) Early Bronze Age trephined cranium.

Adult male, Beaker burial, from a Dorsetshire round barrow: cranial vault unusually thick, simulating Paget's disease. Large left parietal fragment excised. Absence of bony healing coupled with the finding of the fragment *in situ* suggests an immediately fatal issue.

(3) T. NICOL: (*a*) Preparations showing the distribution of fatty substances and glycogen in the uterus of the ferret during the oestrous cycle.

"Fat" stained by Sudan III was found to be absent from the endometrium of the uterine horns during the follicular phase of the cycle and in the earlier stages of pseudo-pregnancy: it first appears in the uterine epithelium about the 31st day of pseudo-pregnancy and markedly increases 1 week later. At this stage a considerable amount of degeneration occurs throughout the endometrium, but the stainable "fat" is not necessarily associated with this degeneration.

Large rounded or oval cells with brownish granules are found in moderate numbers in the endometrial folds, especially about the middle of the follicular phase of the cycle during the first 3 weeks of pseudo-pregnancy. Later in

pseudo-pregnancy they are practically absent. The granules usually obscure the nucleus and assume a deeper brown colour with Sudan III: they stain but slightly with osmic acid and are visible in unstained sections. In ferrets injected with trypan blue, the bodies of some of these cells are faintly stained with the vital dye. These cells closely resemble the "fat carriers" described by the author in the endometrium of the pregnant guinea-pig (*J. Anat., Lond.*, vol. LXXII, p. 618).

Glycogen was found in small amounts in the cells lining the glands during the early part of the follicular phase of the cycle, in moderate amounts during the second half of the follicular phase, and in small amounts at the 10th day of pseudo-pregnancy. Towards the end of pseudo-pregnancy, when fat was abundant, no glycogen was present. The amount of glycogen varied in different animals at the same stage of the cycle.

(*b*) Preparations showing the results of intravitam staining with trypan blue in the pseudo-pregnant uterus of the ferret.

Ferrets injected with trypan blue were killed at seven different intervals between the 10th and 40th day of pseudo-pregnancy. In all the animals practically similar results were obtained. In the endometrium very few large cells with granular vital staining were seen; the epithelium showed no vital staining; the fibrous septa in the endometrial folds were diffusely stained with the dye and showed a moderate number of small, elongated, vitally stained cells. In the muscular layers and around the large blood vessels a considerable number of large and small cells were found showing moderately intense granular vital staining. The surface peritoneum was also vitally stained.

These results agree with the author's previous findings in the guinea-pig.

SOME OBSERVATIONS ON THE LIVER OF THE PIG: THE HEPATIC LOBULE AND LIVER CELL DURING POST-NATAL GROWTH

By E. G. WHITE

Research Institute in Animal Pathology, Royal Veterinary College, London

Since the first description of the hepatic lobule in the pig by Wepfer in 1664 and in a number of animals and man by Malpighi two years later, the pig's liver has served as the classical example of the mammalian liver. The well-developed interlobular connective tissue of the liver of the adult pig makes it particularly suitable for a study of the hepatic lobule by dissection and reconstruction. The valuable work of Kiernan (1883) on the architecture of the liver was followed by the conception of "biliary lobules" (Sabourin, 1888), units having a bile duct as their centre, as opposed to the central vein of the hepatic lobule. This structural unit was difficult to define and the theory found little support among histologists. The "portal structural unit" of Mall (1906), although invaluable as a physiological conception, is not easy to define anatomically. In animals with well-developed interlobular connective tissue—the wild and domestic pig, camel, dromedary and American bear—the original hepatic lobule is the only anatomical unit which appears to be applicable. In the seal, the animals in which Mall saw the clearest anatomical expression of the "portal unit", there exist both hepatic and portal lobules: the opinion formerly held that the latter are bounded by connective tissue has been shown to be incorrect (Arey, 1932).

Among the most important papers dealing with the pig's liver are those of Illing (1905), Theopold (1910), Kretzschmar (1914) and Johnson (1917, 1918 *a*, *b*, 1919). Johnson was apparently unaware of Kretzschmar's work, and the latter makes no reference to the paper by Theopold. As a result of such omissions and the incorrect use of measurements of the diameter of hepatic lobules in computing their volume (Kretzschmar, 1914), there still exists considerable doubt as to the size and number of the liver lobules of the pig during growth and as to the process which brings about increase in the size of the liver after birth.

In the course of an investigation into experimental cirrhosis, the opportunity was taken of studying these problems on a number of normal pigs. Some of the animals were derived from two litters of seventeen and eight pigs respectively, farrowed and reared at the Institute. In addition, samples of liver from fifty pigs of varying age were received in fixative from various sources.

Two of the normal pigs formed part of a group of five animals on which repeated partial hepatectomies were performed at intervals under nembutal

anaesthesia, approximately 5 g. of liver being removed at each operation. From these animals it was possible to make observations on two or three liver samples taken at different ages and, finally, to examine the liver post-mortem. An account of the work on cirrhosis will be published later.

As far as possible, liver samples were always obtained at the same time of day in order that variations due to feeding might be reduced to a minimum. The liver sample was fixed within 10–15 min. of removal from the animal in formol saline and "susa"; in most cases Zenker and Bouin were also used. Animals were slaughtered with the humane killer or by stunning followed by bleeding. For the purpose of comparison with the results of other workers, all measurements of lobules, liver cells and nuclei were made on formalin-fixed material, embedded in paraffin by Peterfi's methyl benzoate celloidin method. Sections were cut at 8μ.

THE HEPATIC LOBULE

Shape

Although the liver lobules of the pig are commonly described as five- or six-sided polyhedra of a fairly definite size, the variation in the same liver is very great, and the largest lobules may be five or six times the volume of the smallest ones. Those on the surface are usually fairly regular in shape, being pyramidal with a hexagonal base which is slightly convex and situated immediately beneath Glisson's capsule, and having an apex from which emerges the central vein. The illustrations in the paper by Johnson (1918*b*) give an excellent picture of the isolated liver lobules and one which we are able to confirm. In the deep lobules the central vein may leave at any position, and the lobule usually conforms to no regular geometrical shape. In the course of measurements of lobule diameters in histological sections, the shape of the lobules (only those cut transversely through the central vein were included) was as follows:

No. of sides	No. of lobules
3	1
4	66
5	308
6	246
7	28
8	1

Thus, in cross-section, almost all the lobules have five or six sides, the pentagonal cross-section being rather more common. No evidence was obtained of any change in shape of the lobule during growth; in histological sections and in macerated specimens the shape was similar in animals of all ages, the length and breadth being approximately in the ratio 3:2.

Size

Illing (1905) gives the diameter of the hepatic lobule as 1573μ in the adult pig, and 872μ in animals described as "young". Ellenberger (1911) and Ellenberger & Schumacher (1914) give 1–1·5 × 1·5–2·5 mm. as the average

dimensions of the lobule in the adult. In the piglet, the diameter is given as 0·87 mm. For the human liver, Pfuhl (1932) gives 1003–1534μ for the adult (mean = 1180μ), and 649–1534μ (mean = 885μ) for a 1-year-old child.

Reference to previous work shows that considerable differences exist between the values for animals said to be of the same age given by different authorities. These are probably explained in part by differences in liver weights, and the weight of the liver as well as the age of the animal should always be given. In addition, the difficulties in measuring the diameter of a lobule in cross-section are considerable, the mean of three diameters being used by most workers.

Personal observations.

Sections were stained with Hansen's iron haematoxylin and counterstained with picrofuchsin. They were projected by means of an Abbé drawing apparatus on to thick foolscap paper at a magnification which varied from ×50 to ×200 and so arranged that eight to twelve lobules were traced on each sheet. In this way the amount of paper used was in each case kept within narrow limits. It was found necessary to weigh each sheet of paper before use as the weight of individual sheets from the same ream varied from 5·133 to 6·196 g. Fifty lobules were traced from each pig, using at least three large sections taken from different parts of the liver (except in the case of small hepatectomy samples, when this was not possible). Only lobules which were cut in a plane approximately at right angles to the central vein were traced and care was taken not to trace the same lobule more than once. The lobules were cut out and accurately weighed. The mean area of cross-section of a lobule could be readily calculated from the weight of the sheets and of the lobule tracings, the area of the sheets, and the magnification used. In order to obtain an index of the size of a lobule, it is clearly more accurate to measure the area of cross-section of a lobule than the diameter. The mean diameter of the lobules was then calculated from the mean area of cross-section assuming them to be regularly hexagonal.

$$\text{Diameter of regular hexagon} = \sqrt{\left(\frac{\text{Area}}{6}\,\frac{16}{\sqrt{3}}\right)}$$
$$= 1{\cdot}24\sqrt{\text{Area}}.$$

The results of thirty-five observations are shown in Table I. It will be seen that in some of the animals more than one estimation was made using hepatectomy samples: this is indicated by, for example, pigs 11 *a*, *b*, *c* and *d*. The observations are arranged in order of liver weight, actual or estimated. In the case of hepatectomy samples, the animal's liver weight was estimated from its live weight at the time of operation and the liver weight subsequently obtained at autopsy.

From Table I it will be seen that the lobule diameter increased from 370μ at 5 days after birth to 1550μ at 297 days. Two livers show even higher values than the latter and, had the animals been allowed to survive longer, liver weights of 3000 g. might well have been obtained. As these animals formed the normal controls of an experiment on cirrhosis, it was not possible to prolong the observations beyond 297 days.

Table I. *Size and number of liver lobules*

No. of pig	Age days	Liver weight g.	Projection method: Lobule diameter mm.	Projection method: Lobule volume c.mm.	Projection method: No. of lobules	Maceration method no. of lobules
Routine 50	5	30	0·37	0·08	$0·35 \times 10^6$	—
Litter 1, 4	7	55	0·37	0·08	$0·64 \times 10^6$	—
Routine 2	46	95	0·60	0·34	$0·26 \times 10^6$	—
Litter 1, 14	20	150	0·56	0·27	$0·51 \times 10^6$	—
Litter 1, 12	45	170	0·61	0·35	$0·45 \times 10^6$	$0·33 \times 10^6$
Routine 24	24	175	0·58	0·30	$0·54 \times 10^6$	—
Routine 1	46	190	0·70	0·54	$0·33 \times 10^6$	—
Routine 19	65	220	0·73	0·62	$0·33 \times 10^6$	—
Litter 2, 4	25	275	0·61	0·36	$0·71 \times 10^6$	—
Litter 2, 3*a*	35	400*	0·67	0·47	$0·79 \times 10^6$	—
Litter 2, 1*a*	35	400*	0·97	1·45	$0·26 \times 10^6$	—
Litter 1, 17	61	560	1·16	2·47	$0·21 \times 10^6$	—
Litter 1, 5	62	620	0·90	1·16	$0·50 \times 10^6$	—
Litter 1, 23	66	735	0·85	0·97	$0·70 \times 10^6$	$0·73 \times 10^6$
Litter 1, 18	66	745	1·08	1·98	$0·35 \times 10^6$	—
Abattoir 2	—	840	1·14	2·37	$0·33 \times 10^6$	$0·36 \times 10^6$
Abattoir 7	—	860	1·28	3·30	$0·24 \times 10^6$	$0·25 \times 10^6$
Abattoir 5	—	1160	1·11	2·23	$0·48 \times 10^6$	$0·41 \times 10^6$
Litter 1, 19*a*	81	1350*	1·46	4·88	$0·26 \times 10^6$	—
Litter 2, 7*a*	98	1350*	1·11	2·12	$0·59 \times 10^6$	$0·39 \times 10^6$
Abattoir 1	—	1375	1·36	3·97	$0·32 \times 10^6$	$0·57 \times 10^6$
Litter 1, 75	86	1430	1·20	2·74	$0·48 \times 10^6$	$0·73 \times 10^6$
Litter 1, 16*a*	86	1450*	1·06	1·86	$0·72 \times 10^6$	—
Litter 2, 3*b*	135	1480	1·15	2·38	$0·58 \times 10^6$	$0·51 \times 10^6$
Litter 1, 11*a*	86	1500*	1·33	3·67	$0·38 \times 10^6$	—
Litter 1, 19*b*	89	1515	1·34	3·78	$0·37 \times 10^6$	$0·69 \times 10^6$
Abattoir 3	—	1570	1·23	2·91	$0·50 \times 10^6$	$0·71 \times 10^6$
Litter 1, 11*b*	125	1800	1·36	3·97	$0·42 \times 10^6$	—
Litter 1, 22*a*	123	2000*	1·21	2·80	$0·66 \times 10^6$	—
Litter 2, 7*b*	164	2000*	1·59	6·06	$0·31 \times 10^6$	$0·45 \times 10^6$
Litter 2, 7*c*	239	2130	1·50	5·33	$0·37 \times 10^6$	$0·42 \times 10^6$
Litter 1, 16*b*	125	2150*	1·25	3·09	$0·64 \times 10^6$	—
Litter 1, 11*c*	229	2400*	1·54	5·76	$0·39 \times 10^6$	$0·48 \times 10^6$
Litter 1, 11*d*	297	2550	1·55	5·97	$0·40 \times 10^6$	$0·50 \times 10^6$
Litter 2, 1*b*	160	2650	1·60	6·21	$0·40 \times 10^6$	$0·37 \times 10^6$

* Estimated from body weight.

	Projection method	Maceration method
Mean no. of lobules	$0·45 \times 10^6$	$0·49 \times 10^6$
No. of observations	35	16
Correlation coefficient (no. of lobules: liver weight)	0·00	0·11
Standard error of correlation coefficient	0·17	0·26

The increase in diameter of the lobules indicates an increase in volume, assuming that the shape of the lobule does not alter, of $\times\left(\frac{1550}{370}\right)^3$, i.e. 73·6 times. The liver weight increased from 30 to 55 g. to 2550 g., i.e. from 85 to 46 times. In Kretzschmar's (1914) series of observations the ratio of largest to smallest liver weights is $\frac{3100}{27}$, i.e. $\times 115$; that of the hepatic lobule volume is $\left(\frac{1550}{330}\right)^3$, i.e. $\times 104$, He failed, however, to realize this close relationship for, dealing with the first two observations in his table, he stated: "Es hat nämlich der Grössendurchmesser in den ersten 14 Tagen um nahezu das Doppelte

zugenommen. Dies entspricht aber einer Vervierfachung des Inhaltes eines Leberläppchens, nicht etwa nur einer Verdoppelung", whereas, in fact, it indicated an eightfold increase in volume!

The increase in cross-section of the lobules during growth is illustrated in Pl. I, figs. 1–4.

Number of lobules

That the hepatic lobules in man and mammals increase in size during growth has long been known, but the question as to whether this increase is sufficient to account for the growth of the liver has not been decided. Illing (1905) suggested that the increase in size of the liver might result from the formation of new lobules, enlargement of pre-existing ones, increase of interstitial tissue, or a combination of these. He did not find any evidence of the new formation of lobules. The age and liver weight of the animals were not given. Mall (1906) gave an approximate estimate of the number of lobules in the dog's liver based on the area of surface lobules and the volume of the liver and, as a result of numerous calculations, he considered 480,000 to be the probable number. This rough estimate, unsupported by maceration experiments, proved to be remarkably close to the values subsequently obtained by Johnson (1918*b*).

Mall's measurements of lobules in foetal pig livers appear to have related to structures different from the hepatic lobules of post-natal life. We have been unable to distinguish definite hepatic lobules in piglets earlier than 5–7 days after birth, when they become outlined by the peculiar orientation of the peripheral liver cells. The development of any collagenous connective tissue septa takes place much later. No foetal livers were included in our series. Mall considered that the lobules can increase in number throughout life, even when completely surrounded by collagenous septa, and he gave diagrams illustrating the formation of new lobules by binary fission.

Theopold (1910) was of the opinion that multiplication of lobules could take place up to 6 weeks after birth, by which time they are usually surrounded by collagenous septa. From this time, increase in size of the liver was considered to be due solely to increase in the size of the lobules. Ellenberger (1911) was unable to find evidence of the new formation of lobules. Kretzschmar (1914) recorded the occurrence of twin lobules in pigs a few days old and stated that these are less frequently seen in the adult. He believed increase in size of the lobules to be the most important factor in producing growth of the liver and found no evidence of multiplication of lobules.

Johnson (1919) described in detail the process of lobule multiplication by binary fission, the central vein first dividing, followed by ingrowth of the collagenous interlobular septa resulting in more or less complete separation. Unlike Mall, he did not suggest that the capillary length of the sinusoids within the lobule remained the same during growth and indeed his observations on the increase in size of the lobule during growth made this hypothesis untenable.

His measurements were restricted to livers weighing 1132–1942 g. On p. 321 of his article he states: "Formation of lobules begins at an early age and, I believe, continues throughout all stages to the adult." Clara (1931*a*) agreed with Johnson's views on lobule multiplication and suggested that, when the liver cell columns and capillaries reach a certain length, lobule division is stimulated. Further subdivision before separation is complete leads to the formation of compound lobules, the process being initiated by division of the central lobular vein.

Using Kretzschmar's figures correctly, Pfuhl (1932) showed the surprising agreement between increase in size of the lobules and of liver weight. In view of the variations in blood content of the liver, specific gravity, etc., he considered that the figures showed that the lobules do not increase in number after birth, but only in size.

Personal observations.

(*a*) *Direct counting of liver lobules.* Liver tissue fixed for 3–5 days in formol saline was cut into rectangular blocks of such a size that they might be expected to consist of an approximately equal number of lobules e.g. from 0·3 g. in the case of small livers, to 3–4 g. in large livers. Except in the case of hepatectomy samples, which weighed only about 5 g., two or three blocks of tissue were used. The blocks were rapidly blotted, suspended by a silk thread and weighed to the nearest milligram. They were then placed overnight in a stoppered bottle containing 50–100 c.c. of 50 % hydrochloric acid (Johnson, 1918*b*). In the morning, the bottles were placed in an oven at 60° C. for an hour, when the blocks were removed and the surface lobules were counted under a hand lens or, in the case of very small livers, under a stereoscopic binocular microscope. Care was taken to avoid counting the same lobules on more than one side of the block. The blocks were replaced in the acid and left at 60° C. until gentle shaking produced slight separation of the lobules. This happened 2–6 hr. after replacement in the oven. The blocks and acid were then poured into large flat white dishes and 3–4 parts of water were added. Under a lens or low power microscope the blocks were carefully separated into their component lobules with blunt mounted needles. Very little teasing was necessary, except when separating compound lobules into their component parts.

Half the number of surface lobules was deducted from the total number of lobules, giving the approximate number of complete lobules in the block of tissue. From the weight of the block and of the whole liver, the total number of lobules was calculated. It was found that, within wide limits, the specific gravity of a pig's liver fixed in formol saline was the same as that of the fresh liver—1080. No correction was therefore necessary.

The results of sixteen counts are included in Table I. The animals denoted by "Ab." were slaughtered at the abattoir and their age is not known, though from the liver weights they were probably 4–6 months old. The difference between duplicate estimations made on the same liver varied from 2 to 14 %.

(*b*) *Estimation of total number of lobules from area of cross-section.* The mean volume of the liver lobules was calculated, using the data already obtained for their mean area of cross-section (Table I), assuming them to have a uniform theoretical shape. Our own observations and those of Ellenberger (1911) have shown that the average liver lobule is one and a half times as long

as it is broad and this relation was found to apply to pigs of varying age. The mean volume of a lobule was calculated from the formula

$$\text{Volume} = A \times \tfrac{3}{2}\sqrt{A},$$

where A is the mean area of cross-section already mentioned. It must be pointed out that this shape is purely arbitrary and any other might have been chosen, in which case the results would be proportionately greater or less than those given here. The total number of lobules was calculated from the liver weight—actual or estimated—after allowing for the shrinkage occurring during fixation, dehydration and embedding.

Measurements of the weight of blocks of liver tissue in the fresh state and again after fixation, dehydration and embedding in paraffin showed that the total shrinkage of volume in the process was approximately 50 %. (The block was weighed in air and water or some other suitable fluid at each stage of the process.) The diminution in area of one side of a rectangular block was 30 %, the decrease in one edge was 20 %. (The use of Peterfi's methyl benzoate celloidin method after formol fixation probably resulted in less shrinkage than would have taken place with other fixatives.)

The volume of the lobule calculated from the area of cross-section was therefore doubled to give the volume in the fresh state. A further correction for the specific gravity of the fresh liver—1080—was also applied.

Estimations were made in this way on thirty-five samples and the results are given in Table I. It will be seen that the liver weight varied from 30 to 2650 g. and the age of the animals from 5 to 297 days. In animals less than 5–7 days old, the lobules could not be outlined with sufficient accuracy for tracing, and the maceration method was not successful with animals less than 45 days old.

The results of the estimations of the total number of liver lobules are shown graphically in Chart 1. In this chart, both series of observations are arranged on either side of a line parallel to the abscissa, i.e. there appears to be no tendency for an increase in the number of lobules between the first and last observations. It is realized that too few observations were made on very small livers to allow of definite conclusions at the extreme left of the chart, but it would seem that, if multiplication of lobules occurs later than the first few days after birth, it is of little importance in the growth of the liver. Its presence would be shown by an upward slope of the points from left to right. Since no such slope is seen, increase in size of the lobules appears to be sufficient to explain the growth of the liver. The statistical data given at the foot of Table I are in accordance with this view. Calculation of the coefficient of correlation necessitates the assumption that the relation between liver weight and number of lobules can be represented by a straight line. The standard error of the correlation coefficient is in both series greater than the correlation coefficient; this makes it highly improbable (Bradford Hill, 1937) that there is any relation between the two characters, liver weight and number of lobules.

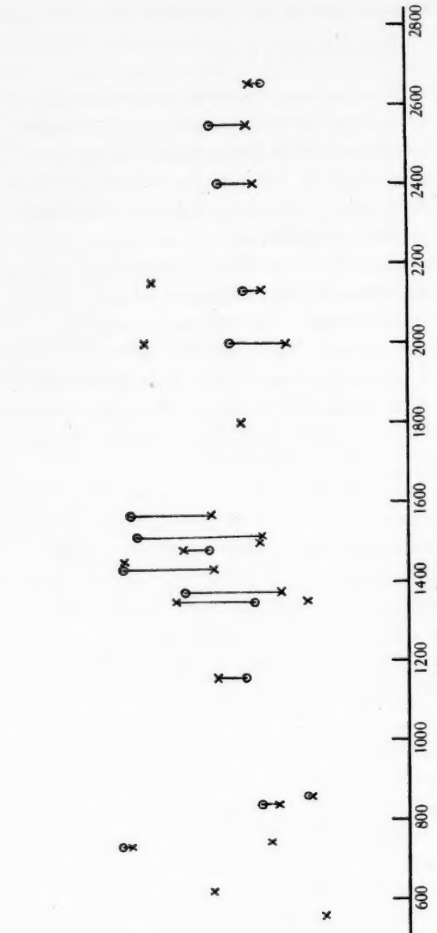

Chart 1. Relation between liver weight and number of hepatic lobules.
× Projection method. ⊙ Maceration method.
Values for the same liver sample are connected by a vertical line.

Grouping the observations obtained by projection according to the liver weights, as shown in Table II, affords further evidence against any significant increase in the number of lobules, as the means of these groups are unaffected by increase in liver weight.

Table II. *Liver lobule counts (projection method) grouped according to liver weight*

Liver weight g.	No. of observations	Mean no. of lobules
0–	7	$0{\cdot}44 \times 10^6$
200–	5	$0{\cdot}46 \times 10^6$
600–	5	$0{\cdot}42 \times 10^6$
1000–	4	$0{\cdot}41 \times 10^6$
1400–	6	$0{\cdot}51 \times 10^6$
1800–	8	$0{\cdot}45 \times 10^6$

If the increase in size of the liver were due solely to increase in the number of lobules, an increase of about eighty times would be needed. That this does not occur is evident from the very great increase in size of the lobules which takes place. We have shown that the liver lobule, between five and 297 days after birth, increases in volume from 0·08 to 5·87 c.mm., i.e. 73·4 times; during this time the liver weight (proportional to its volume) increases from 30 to 55 g. to 2550 g., i.e. from 46 to 85 times. Similar calculations can be made taking any two values for liver weight and lobule diameter. Thus, allowing for experimental error, the increase in volume of the lobule appears to be sufficient to account for the gain in weight of the liver. Formation of lobules probably occurs during the first few days after birth and may perhaps continue until the interlobular connective tissue septa are laid down, first in pre-collagenous reticulum, then in collagen. Evidence of this formation of new lobules in very young animals persists in older animals in the form of compound lobules, divided more or less completely by collagenous septa. We cannot agree that the presence of these compound lobules in adult pigs is evidence of their recent formation. Examination of histological sections and of macerated specimens has shown no significant increase with age in the number of compound lobules; this should be demonstrable if they are formed in considerable numbers. In sections, from 3 to 7 % of lobules were found to be compound in animals more than 30 days old. They are usually composed of two, occasionally of three, lobules. In macerated livers, the compound lobules were composed of two to six lobules fused to very varying extent but comparatively easily separated by dissection with blunt needles under a lens or low-power microscope.

Interlobular connective tissue

The development of reticulum will be dealt with in detail in a later paper on cirrhosis in the pig.

In a 254 mm. embryo, Johnson (1919) recorded the first evidence of definite hepatic lobules, demarcated by the arrangement of the liver cells in columns stretching between portal tracts, and by the spacing of branches of the hepatic

veins at equal distances from branches of the portal vein. On the other hand, he mentioned that livers from pigs 2 hr., 24 hr. and 3 days after birth showed no advance in lobular structure. A condition as advanced at birth as that described by Johnson (1919) would appear to be the exception rather than the rule. If clearly marked lobulation is present before birth, the theory that its origin is due to the negative pressure in the pleural cavity transmitted through the right auricle to the vena cava and hepatic veins is incorrect (Pfuhl, 1932).

Theopold (1910) found no definite lobules in the foetus or new-born pig. From 3 to 7 days after birth, the cells around the branches of the hepatic veins became radially arranged and the number of portal vessels increased. Variation in the degree of lobulation in individual animals was very marked, a 7-day-old pig showing no further development than one $4\frac{1}{2}$ days old. Johnson (1919) also recorded the first appearance of radial columns of liver cells 4 days after birth.

We have examined the livers of seven pigs between birth and 7 days. The first indication of a lobular pattern was found at 5–7 days due to a columnar arrangement of the liver cells between small branches of the portal vein. In some sections, shrinkage had resulted in separation into lobules along these columns. At the same time, the central cells of the lobule began to arrange themselves in radial columns (Pl. I, fig. 2). The first evidence of outgrowth of collagen fibres from the portal tracts was found by Johnson (1919) at 3–4 weeks. In our series of animals, 20–24 days marked the beginning of this outgrowth. In one animal, however, the lobules were almost completely surrounded by collagen at 25 days, but this was exceptional. The clearly marked lobulation seen in Pl. I, fig. 3 was due to thick bundles of reticulum surrounding the lobules; transformation into collagen had hardly commenced at this age (20 days).

In three pigs (litter-mates) 35 days old, the variation in development of connective tissue was very striking. In one animal, almost every lobule was completely surrounded by a thin band of collagen, in the second, the bands extended for only one-half to two-thirds of the distance between portal tracts, whilst the third showed almost no outgrowth of collagen.

The development of the collagenous septa commences as single wavy collagen fibres growing outwards from the portal tracts and eventually overlapping at the midpoint between them. The thickness of the septum increases until in the adult it is formed of several collagen fibres. In our experience, the collagenous septa are always completed by 60 days after birth, usually between 45 and 60 days, but occasionally earlier. Theopold (1910) gave 6 weeks as the usual time for completion of the septa.

Attempts to estimate in sections the relative amount of connective tissue and liver parenchyma can give only very approximate results. Pfuhl (1932) estimated the amount of portal and interlobular connective tissue (including the walls and lumen of vessels) in sections stained with picrofuchsin by tracing and cutting out and weighing the connective tissue. In a 9-month-old pig, he

obtained a value of 7·5 %, a figure similar to that found in the rabbit. At first sight it may appear surprising that the proportion is similar in the two species but, whereas in the rabbit the portal tracts are very large, in the pig they are small. Kostorz (1936), using a similar technique, obtained figures of 11·6 % for the domestic pig and 5·1 % for the wild pig. She suggested that domestication resulted in an increase in connective tissue. The age of the animals used was not given.

We have carried out four estimations using the same technique, with results as follows:

Age of pig days	Interlobular connective tissue %
0	4·5
46	7·6
120	8·8
297	9·5

The value obtained for the adult pig is intermediate between those of Pfuhl and Kostorz. Such estimations give no indication of the volume of connective tissue in relation to parenchyma, but merely express the proportion seen in sections.

The liver cell

According to Theopold (1910), the liver cells in the foetal pig are polyhedral, sometimes with two nuclei, and measure 9·6–18·5μ in diameter. Ellenberger and Schumacher (1914) gave 20–25μ as the diameter of the liver cells in the adult; in the young pig they are stated to be 2–7μ smaller. Illing (1905) measured 100 liver cells and found the diameter to be 17·9–27·2μ (mean = 21·4μ) in the adult and 10·2–19·6μ (mean = 14·6μ) in the piglet (age not given). He gave similar measurements for the other domestic animals but the difference in size between the cells of adult and young animals was less than in the pig. Since the difference in diameter between the liver lobules in the adult and young pig was approximately 100 %, the increase in size of the cells was almost sufficient to account for it.

In a 12-hr.-old pig Kretzschmar (1914) gave 8·8–17·5μ (mean = 13·6μ) as the cell diameter, while the values for the adult were 17·5–29·6μ (mean = 23·3μ). His series of measurements do not, however, show an uninterrupted increase in cell diameter with age, e.g. the diameter in a 31-day-old pig is given as 13·6μ; during this time, however, the liver lobules have increased from 330μ at birth to 670μ. The increase in size of the lobules cannot, therefore, have been due to enlargement of the liver cells.

Johnson (1919) gave measurements for cell diameters varying from 11·9μ in an 80 mm. foetus to 16·0μ in a 254 mm. foetus, and 19·8μ in the adult pig. During the same time, the liver sinusoids diminished in diameter from 12·9 to 8μ. In a single lobule of a 254 mm. embryo, Johnson estimated the number of liver cells at 21,000: in the adult, he calculated the number to be 465,000. Unlike previous workers, he considered that cell multiplication played an important part in the growth of the lobules.

Using sections stained with Ehrlich's haematoxylin, and a magnification of × 1640, we have traced, by means of an Abbé drawing apparatus, fifty cells and nuclei of nine livers of pigs of varying age. Owing to the irregular shape of many of the liver cells, it was found unsatisfactory to measure their diameter, hence the cell area was measured with a planimeter. Voss (1936) described the use of the planimeter for measuring the area of cell nuclei as "eine neue Methode der Kernmessung"! We have used this method in measuring liver lobules but have found that cutting out and weighing the paper tracings was more rapid and sufficiently accurate.

In order to simplify calculations, we have assumed the cells to be square in cross-section, the diameter (d) being calculated from the formula

$$D = \sqrt{2} \times \sqrt{\text{Area}}.$$

The results are given in Table III.

Table III. *Diameter of liver cells and nuclei*

No. of pig	Age days	Mean nuclear diameter (μ)	Mean cell diameter (μ) $=\sqrt{2} \times \sqrt{\text{area}}$
Routine 4	0	5·9	17·6
Routine 3	0	5·7	18·9
Litter 1, 8	1	6·7	16·7
Litter 1, 14	20	6·3	16·0
Litter 2, 3*a*	35	6·2	17·5
Litter 1, 12	45	6·3	18·1
*Litter 1, 9	61	6·8	15·5
Litter 1, 11*a*	86	5·7	17·8
Litter 1, 11*b*	125	5·9	16·7
Litter 1, 11*c*	229	5·9	19·8

* Received 0·6 c.c. of CCl_4 subcutaneously 24 hr. previously.

It will be seen that, whilst the greatest cell diameter was found in the oldest pig, the next largest was in a new-born pig. The smallest diameter in the normal animals was found in a 20-day-old pig. The difference between the largest and smallest value is small—3·8μ—and is much less than that recorded by previous workers.

In the new-born pig, the liver cells were somewhat rounded in outline, closely packed, and with a very vesicular cytoplasm. Within 12–24 hr. after birth, the size of the cells diminished, the protoplasm became more granular and the cells took on a more polygonal shape. The smallest liver cells are probably present between 24 hr. and 3–4 days, after which a slight increase appears to take place until adult life is reached. It should be noted, however, that the size of liver cells and nuclei varies considerably with the diet and the time of removal of the liver tissue relative to the last meal and with the fixative and method of embedding.

Liver cell nuclei

The diameter of the liver cell nuclei in the pig is given by Illing (1905) as 3–8μ. Theopold (1910) gave 6–8μ for the foetus. In the rat, Plenk (1911) gave 6μ at birth, 1 week and 4 weeks, and 8μ in the adult.

From Table IV it will be seen that the largest nuclei were found in pigs 1 and 20 days old, and this may be due to the frequency of mitoses in these animals. The mean diameter in the adult is seen to be the same as in the two new-born pigs. It should be noted, also, that the ratio of nuclear diameter to cell diameter is not constant.

It is strange that the occurrence of mitoses in the liver of the pig has been seldom recorded. Illing (1905) states: "Im hiesigen Institute (Tierärztlichen Hochschule zu Dresden) sind im Laufe der Jahre Tausende von Leberpräparaten von allen Haustieren untersucht, aber niemals mitotisch Kernfiguren gefunden worden." Ellenberger (1911) makes a similar statement (p. 350): "Ich habe bei wachsenden Tieren keine karyokinetischen Figuren, keine lebhaften Anzeichen einer direkten Zellvermehrung und keine Zeichen einer Läppchenentstehung beobachtet." Although Kretzschmar (1914) did not deny the possibility of mitosis being present in the liver of the pig, he was unable to find evidence of it in animals of any age.

In young pigs, we have experienced no difficulty in finding mitoses in frozen or paraffin sections stained with Ehrlich's haematoxylin and eosin. A group of three mitoses in a growing column of liver cells, for example, was seen in a 2-day-old pig. In new-born pigs there are few mitoses, but these are very numerous during the next few days, to diminish in numbers later, until, in the adult, they are extremely difficult to find. In the rat, Brues & Marble (1937) found not more than one mitosis in 10–20,000 liver cells in the adult resting liver but mitoses were found in more than 3 % of liver cells 48 hr. after partial hepatectomy. In pigs which had received 0·6 c.c. of carbon tetrachloride subcutaneously 48 hr. before slaughter, we have found a mitotic index (number of mitoses per 1000 nuclei) of 6–10. The mitotic indices of thirteen livers are shown in Table IV, in which have been included values for two pigs which had received carbon tetrachloride. The majority of the mitoses were in the prophase stage, but all stages have been observed.

Table IV. *Mitotic index* (*number of mitoses per* 1000 *nuclei*)

Pig no.	Age in days	Mitotic index
Routine 4	0	0·3
Routine 3	0	0·7
Litter 1, 8	1	4·7
Litter 1, 3	2	4·3
Litter 1, 4	7	3·0
Litter 1, 14	20	1·7
Litter 2, 3*a*	35	0·3
Litter 1, 12	45	0·7
*Litter 1, 9	60	6·3
†Litter 1, 17	66	8·7
Litter 1, 20*a*	86	0·3
Litter 1, 20*b*	125	0·5
Litter 1, 20*c*	229	0·0

* Received 0·6 c.c. CCl_4 24 hr. previously. † Received 0·6 c.c. CCl_4 48 hr. previously.

In the rat's liver, Brues & Marble (1937) found the average duration of mitosis to be 49 min. It is clear that, among other factors, the number of

mitoses seen at any time in section will be affected by the time taken for mitosis—the more rapid the process, the less will be the chance of seeing it.

Although we do not suggest that amitosis does not take place in the liver cells of the pig, we have observed no example of it in any normal liver although a number of constricted nuclei have been seen. Brues & Marble (1937) found no evidence of amitotic division in the rat's liver following partial hepatectomy. Whilst Pfuhl (1932) considered that mitosis is the usual method of cell division during normal growth and is occasionally found in the adult liver, Clara (1931*b*) suggested that physiological regeneration takes place by amitosis. In a preliminary communication, however (Clara, 1931*a*), he recorded the finding of numerous mitoses in pigs a few weeks old, the number diminishing rapidly with age. In the horse, Leistner (1936–7) found a single mitosis in a foetal liver and two in an animal 2 years old.

Statistical examination of nuclear volumes was first made by Jacobji (1925, 1926), who measured the volume of 200 liver cell nuclei of the adult mouse. The results were shown graphically as a frequency distribution curve in terms of the cube of the diameter of the nuclei, measured by projection of histological sections. The resulting curve showed three maxima for nuclear volumes, in the ratio 1:2:4. This result was considered to support Heidenhain's protomere theory according to which the volume of cells bears a constant relation to the volume of their nuclei and tends to increase in geometrical progression, i.e. 1:2:4:8.... This theory was expressed by the formula

$$\frac{MK}{MP} : \frac{2MK}{2MP} : \frac{4MK}{4MP}, \text{ etc.}$$

MK being the mass of the nucleus ("Kern") and *MP* the mass of the protoplasm.

A similar method was used by Clara (1927) on the liver of an adult pig, bovine pancreas and the interstitial cells of the testes of two pigs. The results confirmed those of Jacobji and were regarded as further support for Heidenhain's theory. Separate measurements were made on mononuclear and binuclear cells and both graphs showed more than one maximum. The graph for the mononuclear cells is reproduced in Chart 2B, the scale having been altered to facilitate comparison with our own observations given in Chart 2A. Three maxima were obtained for mononuclear cells proportional to 1157, 2328, and 4708 respectively. These numbers represent the cube of the nuclear diameter as measured and are not absolute values. The binuclear cells showed two maxima at 2451 and 4788, their nuclear volumes being the sum of the volumes of the two nuclei, which were usually of almost equal size. It will be seen that the maxima obtained are approximately in the ratio 1:2:4. If the appropriate correction had been applied for the number of binuclear cells (see later), the maxima for these would have been more pronounced, those for the monoclear cells being correspondingly smaller.

Although we have not found any other reference to similar work on the pig,

Jacobji's original work has been followed by an enormous number of papers dealing with the statistical analysis of nuclear size in a great variety of tissues of animals from Monotremes to Primates, and of man. Using an entirely different method, Voss (1928) confirmed Jacobji's results on the mouse liver. Liver tissue was macerated in 1 % hydrochloric acid and the nuclear volumes were calculated from the diameter of the liberated nuclei. Under such conditions, the distinction between the nuclei of liver cells, Kupffer cells and connective tissue cells must have been extremely difficult. Although three maxima were found in the adult mouse, a single maximum only was found in young animals. The number of nuclei measured varied from 462 to 1657. In subsequent work, some authors have drawn frequency curves from measurements

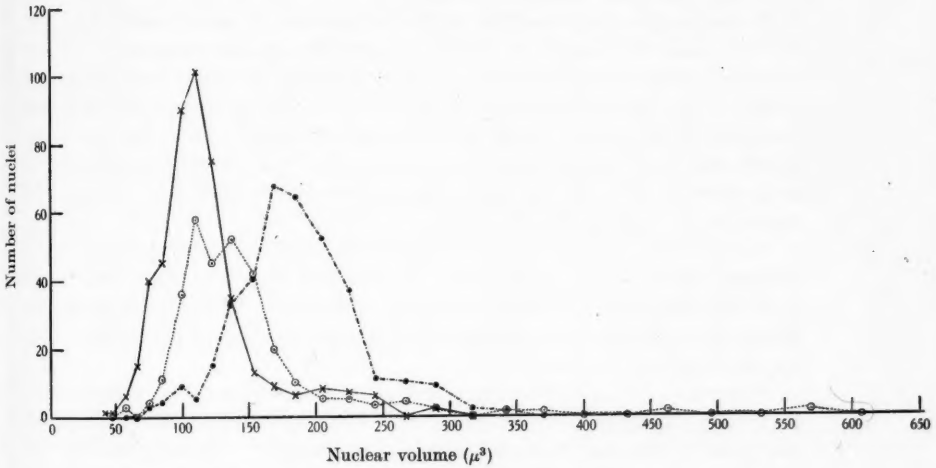

Chart 2 A. Frequency distribution curves of mononuclear liver cells.

●–·–·–● Litter 1, pig 8 (1 day old).
⊙- - - - -⊙ Litter 2, pig. 7*b* (164 days old).
×——× Litter 1, pig 11*d* (297 days old).

of 26–100 nuclei only, maxima on a curve being represented by as few as 3–5 nuclei! Suitable scales for the axes of the charts result in fairly large maxima; from our own observations, we consider they might easily disappear, to reappear at another nuclear volume, if more measurements were made. At least 300–500 nuclei should be measured, as for a Price Jones curve of erythrocytes, and 1000 would be preferable when the significance of small maxima is in doubt.

It is not possible to refer here to more than a few of the most important papers dealing with this subject, but it can be stated that the results obtained for the liver of the mouse, rat and man have been confirmed by several workers. Reference should be made to a valuable paper by Birkenmaier (1934) dealing

with measurements on a large variety of animal species and summarizing in tabular form the results previously recorded. Projection of stained histological sections and measurements of nuclear diameters either with a micrometer eyepiece or after tracing is the method usually employed. More recently, a number of workers have measured the areas of nuclei with a planimeter.

There appears to be fairly general agreement that the presence of more than one maximum is a characteristic of adult animals and appears to be limited to animals higher in the evolutionary scale than the Chiroptera (Birkenmaier, 1934). It is possibly related to the presence of the hepatic lobules characteristic of higher animals. In the chick and adult fowl, a single maximum only has been found (Keller, 1933).

It has become customary to denote the maxima by the symbols $K1$, $K2$, $K3$, etc., and the maximum which contains the greatest proportion of the nuclei is usually printed in heavy type ("Regelzell"). Some workers prefer to denote the smaller maxima as $K1/2$, $K1/4$, etc. The smallest value of K was obtained in the rabbit erythroblast (Freerksen, 1933) and if this is represented as $K1$, the largest maximum, in the cells of the Gasserian ganglion, will be denoted by $K9$. On this scale, the maxima for the liver cell nuclei of the white mouse would be $K5$ and $K6$.

In the adult human liver, Clara (1930) has recorded three maxima, based on observations made on 20 livers. The number of nuclei measured varied from 26 to 230. Jacobji (1935) has examined thirty-six different human tissues. Hintzsche (1936) has given maxima for the syncytial cells of the placenta and for the intestinal epithelium.

The results of an exhaustive study of cell size and growth are contained in a series of papers by Wermel and various collaborators (1932–5) in which the problem has also been studied in tissue culture. Whilst agreeing that definite maxima occur in the nuclei of tissues of many animals and man, they did not invariably find the relationship between them to be in the ratio 1:2:4.... In the human renal epithelium, for example, they obtained a ratio of 1:3 in the histiocytes and fibrocytes of the subcutaneous connective tissue of the hen in tissue culture ratios of 1:3 and 1:13 were obtained (*Mitt.* 1, 1932). In *Anopheles maculipensis*, the ratio of the two maxima was 1:1·5. In their latest communication (*Mitt.* 12, 1935), they record the use of photography in the study of nuclear maxima, photographs of the same nucleus being taken at intervals in tissue culture. Curves giving the change of nuclear volume with time are given and the coefficient of increase in volume was shown frequently to be 0·4 or 0·5. They consider that the evidence available is not in accord with Heidenhain's original protomere theory. It is interesting to note that three amitotic divisions were photographed in tissue culture.

Nuclear size has been found to be affected to a marked extent by diet (Clara, 1935; Schröter, 1937), pregnancy (Biereigel, 1938), experimental hyperclycaemia (Milletti, 1938), prolan administration (Chwatow, 1934–5;

No. and age of pig	19	20	21	22	23	24	25	26	27	28
Litter 1, 11*d*	0	6	12	26	71	134	185	205	138	100
297 days	—	—	—	—	—	—	—	—	—	—
	1	1	6	15	40	45	90	101	75	35
Litter 2, 7*c*	0	1	8	29	47	117	162	109	48	23
239 days	—	—	—	—	—	—	—	—	—	—
	0	3	3	24	47	75	93	84	53	13
Litter 1, 11*c*	0	0	3	8	40	70	59	39	22	4
229 days	0	1	3	9	35	86	75	39	18	4
Litter 2, 7*b*	0	0	1	2	7	16	34	55	98	90
164 days	—	—	—	—	—	—	—	—	—	—
	0	0	3	0	4	11	36	58	45	52
Litter 1, 12	0	0	0	0	1	1	12	16	39	59
45 days	0	0	0	0	0	0	7	23	34	54
Litter 1, 14	0	0	1	10	20	40	49	72	49	34
20 days	0	0	2	3	11	31	40	62	62	48
Litter 1, 3	0	0	1	10	6	22	29	45	44	45
2 days										
Litter 1, 8	0	0	5	2	1	1	6	18	26	47
1 day	0	0	0	0	3	4	9	5	15	33
Litter 2, 6*b**	0	0	0	0	3	2	5	22	59	79
164 days										
Diameter of nuclei (μ) $=2r$	4·3	4·6	4·8	5·0	5·3	5·5	5·7	5·9	6·2	6·4
Volume of nuclei $=\frac{4}{3}\pi r^3$ (μ^3)	43	50	58	67	76	86	98	110	123	137

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Table V. *Frequency distribution of liver cell nuclei*

Diameter of nuclei × 4375 [mm.]

27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
138	100	48	24	11	11	4	9	9	8	0	1	0	1	0
—	—	—	87	58	65	71	32	22	5	2	1	2	0	2
75	35	13	9	6	8	7	6	0	2	0	0	0	0	0
48	23	22	17	13	8	1	0	0	0	0	0	0	0	0
—	—	—	64	39	19	11	6	0	1	2	1	0	0	0
53	13	9	9	4	7	0	1	0	0	0	0	0	0	0
22	4	3	1	1	0	0	0	0	0	0	0	0	0	0
18	4	3	4	9	3	0	0	0	0	0	0	0	0	0
98	90	61	47	13	9	2	2	6	4	1	4	1	1	0
—	—	—	—	—	79	56	72	56	43	46	20	22	6	5
45	52	42	20	12	5	5	3	4	2	0	1	1	0	0
39	59	52	23	7	2	2	0	0	0	0	0	0	0	0
34	54	62	68	33	32	13	6	4	2	0	0	0	0	0
49	34	20	16	4	3	0	2	0	0	0	0	0	0	0
62	48	28	23	3	3	3	1	2	0	0	0	0	0	0
44	45	43	54	54	45	20	14	10	6	5	1	1	0	0
26	47	84	129	85	58	19	12	5	1	0	1	2	0	0
15	33	41	67	64	52	37	11	10	9	2	1	0	0	0
59	79	70	54	13	5	10	7	6	13	5	3	2	0	0
6·2	6·4	6·6	6·9	7·1	7·3	7·5	7·8	8·0	8·2	8·5	8·7	8·9	9·1	9·4
123	137	153	169	186	205	225	246	268	292	317	343	371	400	431

* Received repeated S/C injections of CCl_4: cirrhosis developing.

Table V. *Frequency distribution of liver cell nuclei*

	Diameter of nuclei ×4375 [mm.]																			
26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Total no. of nuclei
205	138	100	48	24	11	11	4	9	9	8	0	1	0	1	0	0	0	0	0	0	1003 all nuclei
—	—	—	—	87	58	65	71	32	22	5	2	1	2	0	2	0	0	0	0	0	347 nuclei >29 mm.
101	75	35	13	9	6	8	7	6	0	2	0	0	0	0	0	0	0	0	0	0	460 mononuclear only
120	48	23	22	17	13	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	605 all nuclei
—	—	—	—	64	39	19	11	6	0	1	2	1	0	0	0	0	0	0	0	0	143 nuclei >29 mm.
84	53	13	9	9	4	7	0	1	0	0	0	0	0	0	0	0	0	0	0	0	425 mononuclear only
39	22	4	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	* 250 all nuclei
39	18	4	3	4	9	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	289 mononuclear only
85	98	90	61	47	13	9	2	2	6	4	1	4	1	1	0	1	0	0	0	0	455 all nuclei
—	—	—	—	—	—	79	56	72	56	43	46	20	22	6	5	2	1	0	1	1	410 nuclei >31 mm.
58	45	52	42	20	12	5	5	3	4	2	0	1	1	0	0	1	0	0	1	0	306 mononuclear only
16	39	59	52	23	7	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	214 all nuclei
22	34	54	62	68	33	32	13	6	4	2	0	0	0	0	0	0	0	0	0	0	338 mononuclear only
72	49	34	20	16	4	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	320 all nuclei
68	62	48	28	23	3	3	3	1	2	0	0	0	0	0	0	0	0	0	0	0	322 mononuclear only
45	44	45	43	54	54	45	20	14	10	6	5	1	1	0	0	0	0	0	0	0	455 mononuclear only
18	26	47	84	129	85	58	19	12	5	1	0	1	2	0	0	0	0	0	0	0	502 all nuclei
5	15	33	41	67	64	52	37	11	10	9	2	1	0	0	0	0	0	0	0	0	363 mononuclear only
22	59	79	70	54	13	5	10	7	6	13	5	3	2	0	0	0	0	0	0	0	358 mononuclear only
5·9	6·2	6·4	6·6	6·9	7·1	7·3	7·5	7·8	8·0	8·2	8·5	8·7	8·9	9·1	9·4	9·6	9·8	10·1	10·3	10·5	
110	123	137	153	169	186	205	225	246	268	292	317	343	371	400	431	463	497	533	570	609	Total = 7565 nuclei

* Received repeated S/C injections of CCl_4: cirrhosis developing.

Wermel *et al.* 1934), ultra-violet irradiation (Wermel *et al.* 1933), various cell poisons, and by growth in tissue culture (Wermel *et al.* 1932).

The only papers dealing with the problem which we have found in English are those by Mainland (1931, 1931–2) on the nuclei of the human ovarian stroma. By statistical analysis, he hoped to introduce quantitative cytological methods which would be of use in pathological conditions affecting the ovary. In the second paper, these hopes had not been realized since no single pathological condition yielded constant results.

Personal observations.

As only a single observation on the pig's liver was found in the literature, it was decided to study the nuclear volume in pigs of different ages, a sufficient number of nuclei being measured to justify the drawing of a frequency distribution curve.

Paraffin sections of formol fixed material stained with Ehrlich's haematoxylin and eosin were projected on to the ground glass plate of a bellows camera fitted on an ordinary horizontal optical bench photomicrographic apparatus. Using a 1/12 apochromatic oil immersion objective and a $\times 12{\cdot}5$ compensating ocular with full bellows extension of the camera, a linear magnification of $\times 4375$ was obtained with a perfectly flat field and extremely sharp outlines. Using an Abbé drawing apparatus, the greatest magnification we could conveniently use was $\times 2000$: that used by previous workers has varied from $\times 1200$ to $\times 2000$. It was found satisfactory to trace the nuclei by transmitted light on fine grain tracing paper placed over the ground glass plate of the camera, the observer controlling the focus by means of a long flexible extension fitted to the fine adjustment of the microscope. An assistant changed the field with a mechanical stage, viewing the field through an aperture at the side of the camera. In this way a large number of fields from several sections of each liver were examined, every liver cell nucleus being traced. No field was traced more than once.

As most liver cell nuclei were circular or oval in cross-section, it was not considered necessary to measure their area with a planimeter. The mean of the greatest and least diameters was used as a measure of the nuclear size. Only nuclei with distinct outlines were traced; nuclei with a blurred irregular contour are seen when the plane of section cuts them unequally.

The results of measurements on 7565 nuclei are shown in Table V. It will be noticed that the values for mononuclear cells are given separately in each case as well as being included with binuclear cells: this procedure is necessary only for the estimation of the relative proportions of mononuclear and binuclear cells. Inclusion of the binuclear cells with the mononuclears does not affect the position of the maxima, but only their relative height since the size of the individual nuclei of binuclear cells is usually the same as for mononuclears. The measurements for binuclear cells are not included, since, owing to the slight difference in diameter between their components, they cannot readily be given in tabular form.

From Table V it will be seen that, in litter 1, pig 11*d*, a count of 1003 nuclei shows a suggestion of a second maximum among the large nuclei; its position, however, is not well defined. Measurements on 347 nuclei of a

diameter greater than 29 mm. (as traced) reveal the position of the second maximum at 32–33 mm. From the volumes of the nuclei given at the bottom of the table it will be seen that the two maxima are approximately in the ratio 1:2. Observations on 460 mononuclear cells also show a second maximum in the same position. The next pig in the table (litter 2, 7*c*) showed no second maximum with 605 nuclei of both kinds, nor did it appear when 143 nuclei of diameter greater than 29 mm. were measured. A count of 425 mononuclear cells does, however, show a second maximum at twice the volume of the first. Each of these pigs showed two maxima when examined at an earlier date—litter 1, pig. 11*c* and litter 2, pig. 7*b* (hepatectomy samples). The only other animal in which a second maximum appeared was one (litter 2, 6*b*) which had received repeated small doses of carbon tetrachloride and was a litter mate of litter 2, pig 7. In this case, it will be seen that the nuclear maxima are further to the right, i.e. the nuclear volumes are greater than in the normal control.

The mean nuclear volume is greater in the young animals than in the older ones. In the case of the 2-day-old pig (litter 1, 3), the maximum is spread over a wide range of nuclear volumes, perhaps owing to the frequency of mitoses at this age.

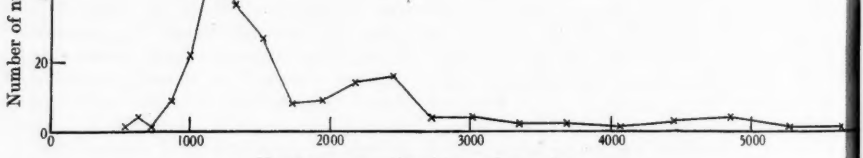

Chart 2B. Frequency distribution curve of mononuclear cells of liver of adult pig. Redrawn to same scale as Chart 2A. Taken from Clara (1927).

Frequency distribution curves of the nuclear volumes of three pigs are shown in Chart 2A. It will be seen that the second maxima are very small when compared with those in Chart 2B, which represents Clara's measurements on an adult pig plotted on the same scale.

In two of the graphs shown in Chart 2A, a suggestion of a very small maximum at half the volume of the "Regelzell" will be seen, though whether this is of significance is not known, but measurements of small numbers of nuclei have frequently led to the appearance of possible maxima which have, however, disappeared when more observations have been made.

In all the observations it is seen that the frequency distribution curve is asymmetrical, being spread out towards the right and very steep on the left. There appears to be a definite limit to the smallest nuclear volume for any tissue, whilst the upper limit extends much further from the mean.

The large magnification we have used gave a range of 19–46 mm. in the diameter of the nuclei. Most workers have had a range of only 8–18 mm.

It is possible that measurements carried out on pigs older than 297 days might reveal more pronounced secondary maxima than those we have obtained. The values of the maxima in the oldest animal correspond closely to those obtained by Clara in an adult pig, although the relative size of the second maximum is considerably smaller and the third, in spite of our having measured 1000 nuclei, failed to appear.

Binuclear cells

When binuclear cells are examined in histological section it is inevitable that a proportion appear to be mononuclear. The proportion recognized as binuclear depends on

(i) The thickness of the section. Sections cut at 4μ show very few binuclear cells as such. A suitable thickness is 8μ.

(ii) The distance apart of the two nuclei. The greater the distance apart the less likely are both nuclei to be included in the same section.

(iii) The angle between the plane of the section and the line joining the centres of the two nuclei.

(iv) The distance on either side of the equator of the nucleus through which it can be sharply focused.

A formula relating these factors has been worked out by Pfuhl (1930) and is very useful in measurements on animals in which binuclear cells are common. For example, a count of 1000 Ascaris eggs, all binuclear, in histological section, showed only 372 as binuclear cells!

The formula usually employed is:

$$m' = \frac{2mq}{2q + 2d + e},$$

where m' = actual number of binuclear cells,

m = observed number of binuclear cells,

q = thickness of section,

d = distance through which focus is sharp, usually taken to be about $0{\cdot}35\mu$,

e = distance apart of nuclear centres; in the pig it was found to be equal to the sum of their radii, since they are usually almost contiguous.

As a rough guide, the number of binuclear cells counted in 5μ sections should be trebled to give the true number and the number in 8μ sections should be doubled. Where the number of binuclear cells is very great, as in the rabbit, the use of the formula is necessary. It has been used by Leistner (1936–7) in the horse where the number of binuclear cells is very small. Of 305 liver cells of a new-born foal, one was found to be binuclear: applying the formula, the true number of binuclear cells was found to be 1·72, and the number of mononuclears 304·28!

The papers by Münzer (1923, 1925) should be consulted for data relating to binuclear cells, though the figures, however, are uncorrected. Pfuhl (1932) has calculated the true percentage of binuclear cells in the rabbit from Münzer's data as 67·3 % as compared with his figure of 20·63 %. Values for other animals given by Münzer are: guinea-pig 18 %, ox 8 %, pig 36 %. In man, Pfuhl (1932) gives 8·3 % for the adult, 1·55 % at birth, and 0·125 % in an 110 mm. foetus. Leistner's corrected figures (1937) for the horse vary from 1·72 % in the foetus and new-born to 10·15 % in animals 18–19 years old.

The percentage of binuclear cells observed in nine pigs of varying age is given in Table VI. The approximate actual number may be obtained in each case by multiplication of the percentage by 2. The increase in the number of binuclear cells with growth is in agreement with the results of others.

Table VI. *Number of binucleate cells (not corrected)*

Pig no.	Age in days	% binucleate cells
Litter 1, 8	1	1·34
Litter 1, 3	2	1·5
Litter 1, 14	20	7·5
Litter 1, 12	45	7·4
Litter 2, 7*b*	164	7·6
*Litter 2, 6*b*	164	12·9
Litter 1, 11*c*	229	8·8
Litter 2, 7*c*	239	11·0
Litter 1, 11*d*	297	10·6

* This animal had received repeated injections of CCl_4.

In the immature mouse, Chwatow & Solowiez (1934–5) found an increase in binuclear cells in the liver following prolan administration. The frequency distribution curve was pushed further to the right and a second maximum appeared which is not normally seen in the immature mouse.

SUMMARY AND CONCLUSIONS

1. The mean area of cross-section and diameter of the hepatic lobule of the pig have been measured by projection of stained histological sections. Thirty-five estimations were made on 26 animals, their age varying from 5 to 297 days and liver weight from 30 to 2650 g. The smallest lobule diameter was 0·37 mm., the largest was 1·60 mm.

2. From the mean area of cross-section of the lobule, assuming a simple theoretical shape, the mean volume of a lobule and the total number of lobules in each liver were calculated. The number varied from 210,000 to 790,000, the mean being 450,000. A direct count of the number of lobules after maceration in hydrochloric acid was made on sixteen of these livers. The number of lobules varied from 250,000 to 730,000, the mean being 490,000.

3. No increase in the number of lobules could be demonstrated later than 5–7 days after birth, this being the earliest age at which either method of estimation could be applied. It is inferred that multiplication of lobules is not of importance later than a few days after birth.

4. No definite hepatic lobules were found in the new-born pig. Their postnatal development is described, with particular reference to the interlobular connective tissue septa. Collagenous septa completely separate the lobules from 45 to 60 days after birth.

5. The difference in size between the liver cell of the new-born and adult pig was found to be considerably less than has been recorded by other workers. The nuclei showed a tendency to decrease in volume with age.

6. Mitoses were easily found in livers of young pigs, being especially numerous between 1 and 7 days after birth. The mitotic index varied in normal pigs from 0·0 in the adult pig to 4·7 in a 1-day-old pig.

7. The results of statistical examination of the volume of liver cell nuclei in nine pigs of varying age are given in tabular and, in part, in graphical form. In the older animals, the frequency distribution curve showed a second maximum at a nuclear volume approximately double that of the first. This second maximum, however, was very small as compared with the findings of other workers in various human and animal tissues.

8. The number of binuclear cells varied from 1·34 % in the new-born pig to 11·0 % in the adult. The actual number of binuclear cells differs considerably from the number observed in histological sections and a method of determining the actual number is discussed.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATE I

Fig. 1. Liver of new-born pig. Closely packed vesicular liver cells. No lobular pattern and few hepatic veins. Groups of erythropoietic cells.

Fig. 2. Liver of 7-day-old pig. Development of hepatic lobules with central lobular veins, surrounded by closely packed liver cells, smaller than in fig. 1 and not vesicular. Periphery of lobule marked by columnar arrangement of liver cells connecting adjacent portal tracts. Erythropoiesis still in evidence.

Fig. 3. Liver of 20-day-old pig. Increase in size of hepatic lobules and well-developed interlobular septa composed of reticular fibres.

Fig. 4. Liver of 297-day-old pig. Only two-thirds of the cross-section of a single lobule is seen. Typical picture of the normal liver of the adult pig with thick collagenous interlobular septa. Sections stained with van Gieson and photographed under the same magnification (×60). Note the increase in area of cross-section of the lobules with age.

Fig. 1.

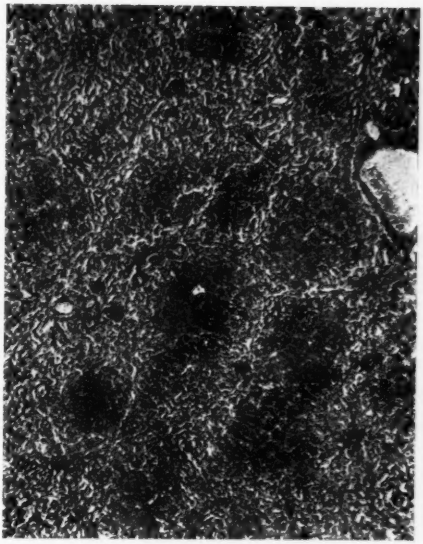

Fig. 2.

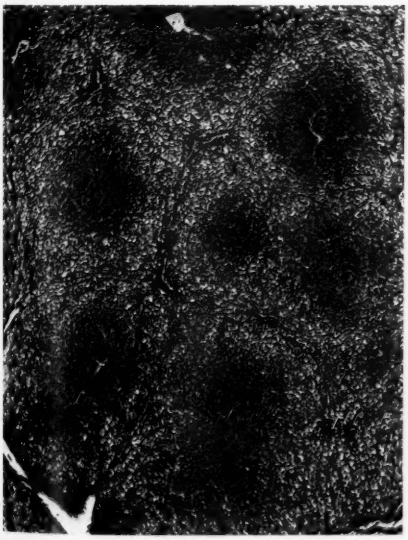

Fig. 3.

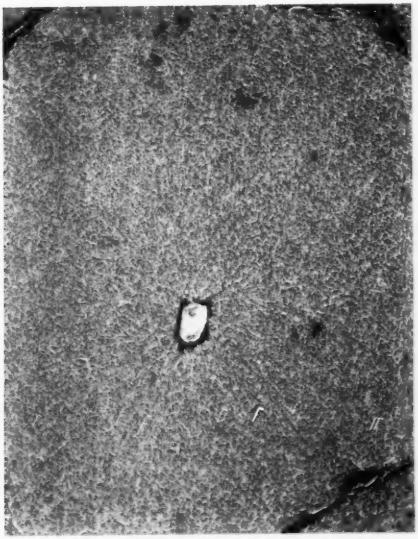

Fig. 4.

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AFFERENT FIBRES OF THE HYPOGLOSSAL NERVE

By C. B. B. DOWNMAN

Sherrington School of Physiology, St Thomas's Hospital, London

For many years doubt has existed as to the precise composition of the hypoglossal nerve. Generally the nerve is stated to consist of motor fibres only, although some authors tacitly assume the presence of fibres subserving "muscle sensibility". In the following paper the present position is indicated, and experiments bearing upon the problem are described.

INTRODUCTION

The greater part of the previous work has consisted of anatomical investigations.

Sensory endings of tongue muscle. The existence of muscle spindles has been affirmed by Franqué (1891, man), Pilliet (1890, cat), Forster (1894, man), Langworthy (1924, cat, dog, rat, opossum), Tarkhan (1936*b*, rabbit). These observations contrast with the negative results of Sherrington (1894), Batten (1897), Cipollone (1897), Baum (1900), Boeke (1927) and Carleton (1937). After recent thorough histological investigation in the rabbit, Boyd (1937) points out that: "There is a rich arborization of nerve terminations in the connective tissue of the tongue, but in the absence of degeneration experiments no statement can be made as to their nature and origin. Nothing resembling a true muscle spindle with perilymphatic space, epilemmal endings and intra-fusal fibres was seen." Boeke (1927) also draws attention to the rich terminal arborization.

Barron (1936) was unable to record action potentials in the hypoglossal nerve of cats, rats and rabbits, even upon stretching, deforming or burning the tongue; nor to the action of destructive acid and alkali. Volleys were set up in the lingual nerve on stretching the tongue, which Barron interpreted as being "proprioceptive" in origin. Corbin & Harrison (1938) recorded no hypoglossal action potentials upon stretching or twisting the cat's tongue, and consider that the lingual volleys are tactile in origin.

Sensory nerve cells. A rudimentary ganglion in line with the sensory root ganglia was first described by Mayer of Bonn in 1833. Although rare in the adult macaque and man, it is present in a wide series of animals and is usually associated with the name of Froriep. Langworthy (1924) finds the ganglion constantly in the cat, although varying in size from a pin-head to a mere sprinkling of cells along the most caudal hypoglossal rootlet. Tarkhan (1936*b*) describes nerve cells situated along the stem of the hypoglossal nerve of the rabbit, at the point of origin of the descending branch; he assumes that these

are sensory cells which have migrated out to the peripheral site. Boyd (1937) and Carleton (1937) do not confirm this finding.

Cervical sensory contribution to the hypoglossal nerve. Sherrington (1894), from his study of an amyelous human foetus, and van der Sprenkel (1924), from anatomical reconstructions of hedgehog embryos, conclude that the second cervical dorsal root ganglion contributes sensory fibres to the trunk of the hypoglossal nerve. Langworthy (1924) found that avulsion of the hypoglossal nerve in cats was followed by chromatolysis of cells in the second cervical ganglion. Corbin *et al.* (1937) found a scattering of degenerated fibres (1–2 % of the 3800–4000 myelinate fibres) in the hypoglossal nerve near the tongue after removal of the second cervical ganglion in the Rhesus monkey. On the other hand, Hinsey & Corbin (1934) were unable to show any contribution of myelinate fibres from the upper four cervical ganglia in cats 10–22 days after ganglionectomy. Tarkhan (1936*b*) also finds no degenerated fibres in the stem of the hypoglossal nerve after section of the anterior rami of the upper cervical nerves in the rabbit.

Experimental results. Langworthy (1924) found no evidence of "pain" fibres upon faradizing the hypoglossal nerve in decerebrated cats, using Sherrington's "pain reaction" as an index. On the contrary, Tarkhan (1936*a*) obtained rises of blood pressure upon faradizing or pinching the nerve in chloralosed cats; stimulation of the central end of a branch of the nerve upon the tongue caused bilateral reflex contraction of the tongue, independent of the lingual nerves. Acheson *et al.* (1936) also illustrate rise of blood pressure and slight contraction of the nictitating membrane accompanying stimulation of the central cut end of the hypoglossal nerve in the urethanized cat.

Conclusion. The evidence relating to sensory endings of tongue muscle is, at its best, equivocal. The evidence concerning afferent fibres in the hypoglossal nerve is equally conflicting, and the existence of such fibres must be regarded as not yet definitely established.

The outstanding problems are therefore clearly defined:

(1) Do afferent fibres of any function run in the hypoglossal nerve?

(2) By what path do they enter the central nervous system?

(3) What is the function and structure of their peripheral endings?

ANATOMY OF THE HYPOGLOSSAL NERVE OF THE CAT

Dissections have shown that the course of the nerves to the tongue and subhyoid muscles in the cat is similar to that described for man. The arrangement found with minor variations in the cat is illustrated (Fig. 1).

The widest variation occurs in the formation of the ansa hypoglossi, from complete separation of n. descendens hypoglossi and n. descendens cervicalis, to fusion of n. descendens cervicalis with the stem of the hypoglossal (Fig. 2).

Certain points of the anatomy of the cat need to be stressed. First, n. descendens cervicalis is usually formed by a branch of the anterior primary

ramus of the first cervical nerve, but occasionally passes off from the loop between the first and second cervical nerves. Secondly, no direct nervous connexion could be found between the upper cervical nerves and the stem of

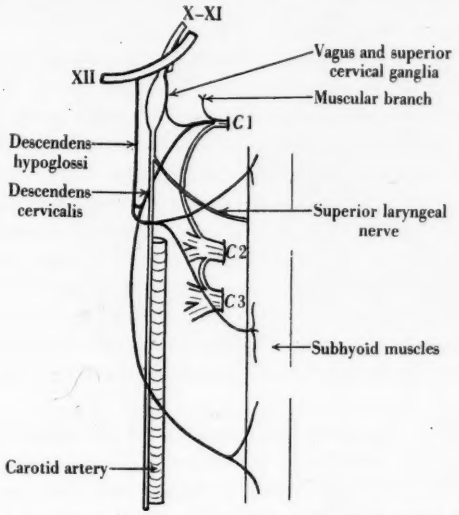

Fig. 1. Plan of the ansa hypoglossi of the cat, as generally found at dissection.

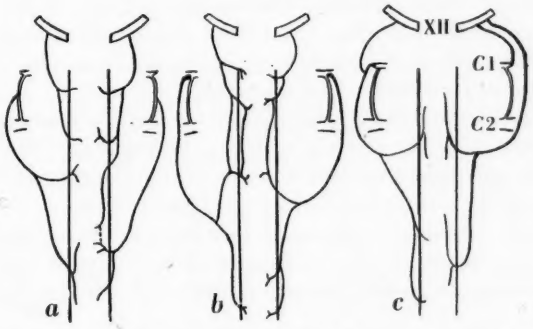

Fig. 2. Variations of the ansa hypoglossi. (*a*) No union between n. descendens hypoglossi and n. descendens cervicalis. (*b*) Ansa formed by wide plexus in the subhyoid muscles. (*c*) No descending branch from the hypoglossal nerve, n. descendens cervicalis uniting directly with stem of hypoglossal nerve.

the hypoglossal. The anterior primary ramus of *C*1 divides into three; one division forms the loop between *C*1 and *C*2, one forms the descending cervical nerve, while the third passes forward into the substance of the neck muscles. In naked eye dissection no fibre has been traced on through the muscle to join the hypoglossal trunk.

METHOD

All the experiments have been acute and performed on cats. The passage of impulses into the central nervous system was indicated by the following reflex actions:

(*a*) Dilatation of the pupil in chloralosed cats.

(*b*) Rise of blood pressure in chloralosed and in decerebrated cats.

The chloralosed cats were prepared by injection of 75 mg. chloralose (Martindale)/kg. body weight intravenously, under preliminary ether anaesthesia. After one hour the pupils are usually slit-like and dilate on stimulation of afferent nerves or end-organs (Bain *et al.* 1935). After the required operative procedure the animal was rested until the pupils were again slit-like and sensitive to a light pinch of the ear. Before decerebration the nerves were dissected out under ether anaesthesia and the internal carotid arteries tied; after decerebration the animal was allowed to rest one hour to blow off the ether. Blood pressure was recorded optically from either the common carotid or femoral artery. Nerves were stimulated faradically by induction coil with silver wire electrodes; also by pinching, whereby any spread of current is eliminated.

Absence of reflex response upon stimulating a nerve may be due to: (1) the preparation being less sensitive in consequence of previous operations, (2) the number of afferent fibres left intact is insufficient, the number of impulses bombarding the nervous centres being too small to affect demonstrably the final motor neurones, (3) all the afferent fibres have been cut. As a precaution against unreliable results due to variations in sensitivity of the animal, observations were made only when the animal was sensitive as judged by the response to a light pinch of the ear. Further, observations were made on both sides, using the contralateral nerve as control.

The blood pressure changes have been recorded as confirmation of the results obtained using the pupil as an index. Irving *et al.* (1937), comparing blood pressure and pupil responses, consider that "dilatation of the pupil due to inhibition of the third nerve nucleus is the more sensitive index of visceral afferent fibres". Personal observation convinces me that dilatation of the pupil is the more sensitive index of somatic afferent impulses also, and abolition of the response is probably due to section of all afferent fibres.

RESULTS

Afferent fibres of the hypoglossal trunk

The hypoglossal nerve was ligated and cut close to its entry into the tongue, m. mylohyoideus being reflected laterally. Stimulation of the central end of the nerve caused wide dilatation of the pupil, rise of blood pressure, and also muscular movements of decerebrated cats (Fig. 3).

These results indicate that afferent fibres are present in the trunk of the hypoglossal nerve. The hypothesis that all of these fibres may enter the central nervous system at the cervical level of the cord was examined. The dorsal root

ganglia of the first and second cervical nerves were removed bilaterally (these ganglia lie outside the vertebral canal and may be removed without exposing

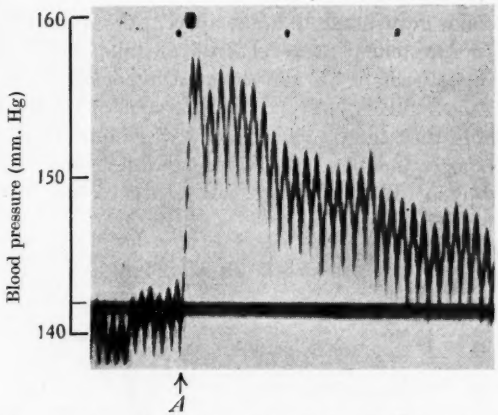

Fig. 3. Rise of blood pressure upon pinching central end of cut right hypoglossal nerve in decerebrated cat. Left common carotid blood pressure; right internal carotid artery tied. Time 30 sec.

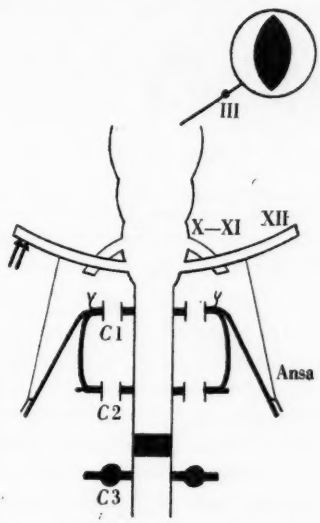

Fig. 4. Dilatation of the pupil upon pinching central end of cut hypoglossal nerve in chloralosed cat, after section of spinal cord at *C*2 and removal of *C*1 and *C*2 dorsal root ganglia.

the cord) and the spinal cord cut under the lamina of the axis. Although all possible cervical pathways were cut, stimulation of the hypoglossal nerve still caused dilatation of the pupil (Fig. 4).

Further, the reflex responses were always abolished by section of the vagus-accessory-hypoglossal group close to the skull (Fig. 5), even though the cervical nerves and spinal cord were intact.

The conclusion is drawn that all of the afferent fibres from the tongue enter the central nervous system at the level of the medulla, and that no fibres pass down to the cervical roots either *via* the ansa hypoglossi or by a direct connexion between the first cervical nerve and the stem of the hypoglossal. It is, of course, possible that the direct pathway was injured. However, such a pathway has not been identified in dissection; while control dissection of the vagus-accessory-hypoglossal group without actual section of the nerves did

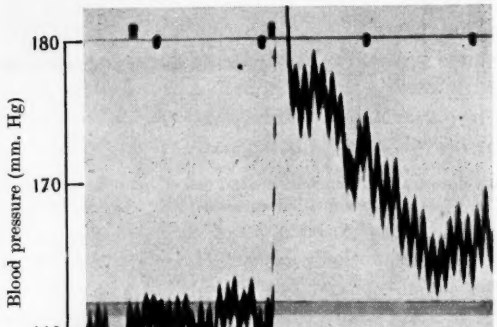

Fig. 5. Rise of blood pressure upon pinching central end of cut left hypoglossal nerve, *B*, in decerebrated cat. No response to pinch of central end of cut right nerve, *A*, after section of right vagus-accessory-hypoglossal group close to skull. Left common carotid blood pressure; right internal carotid artery tied. Time 30 sec.

not abolish the responses. Attempts were made to section only fibres of bulbar origin, through a trephine opening of the base of the skull; the operation caused marked loss of sensitivity of the animal, and as no positive control responses could be obtained the results were of little value.

In certain cats the reflex responses were abolished by section of the vagus-accessory group close to the skull. In others it was necessary to cut also the hypoglossal nerve close to the skull. It appears that the afferent fibres enter with the vagus-accessory group in some animals, while in others some of the fibres enter with the hypoglossal trunk. Removal of the ganglion nodosum, alone or together with the superior cervical ganglion, did not abolish the responses. The ganglion was removed without encroaching upon the junction of the vagus-accessory and hypoglossal nerve trunks.

Afferent fibres of n. descendens cervicalis

When n. descendens hypoglossi was cut, stimulation of the central cut end of n. descendens cervicalis caused pupil and blood pressure responses. These responses were obtained on stimulation of the branches of the nerve close to their entry into the subhyoid muscles; also in those cases where there was no extramuscular union between the descending cervical and descending hypoglossal nerves (Fig. 2). The sensory endings of the afferent fibres therefore appear to lie in the subhyoid muscles.

Removal of the first cervical dorsal root ganglion abolished the reflex responses, whereas removal of the second cervical ganglion had no effect. It is clear that in the cat the afferent fibres of this nerve enter the central nervous system through the first cervical roots. This finding is in agreement with the anatomical origin of the nerve.

Afferent fibres of n. descendens hypoglossi

Stimulation of the central end of the cut nerve caused definite dilatation of the pupil. Attempts to define the course of the fibres in more detail were unsuccessful. The nerve is but a fine strand and rather extensive dissection of the neck was needed in order to establish its identity. Under the experimental conditions the activity of the nerve has proved so ephemeral that responses to stimulation were inconstant and frequently unobtainable, even when marked responses were elicited on first pinching the nerve. The results obtained do indicate the existence of afferent fibres in the nerve. From anatomical considerations it is clear that they travel for part of their course upwards to the central nervous system in the stem of the hypoglossal nerve.

Sensory endings in the tongue

Since stimulation of the hypoglossal nerve close to the tongue causes reflex responses, the endings of the afferent fibres probably lie in this organ. Attempts were made to stimulate such endings in the tongue, using the pupil and blood pressure responses as indices of afferent impulses.

The glosso-pharyngeal nerves were sectioned bilaterally close to the skull and the chorda-lingual nerves sectioned bilaterally proximal to the submaxillary ducts. The jaws being propped open by a gag, the tongue was drawn forwards out of the mouth by a thread through the tip.

No pupil or vascular reflex responses were set up by pinching, crushing, burning the tongue; nor by stretching with tissue forceps to the point of tearing the muscle. Strong faradization through wire electrodes inserted into the tongue caused slight dilatation of the pupil; this response was abolished by section of the hypoglossal nerves bilaterally under mm. mylohyoideus. Since a greater current strength was required to cause dilatation than to set up marked contraction of the tongue, the effect was most probably due to spread of current to pre-terminal nerve fibres.

DISCUSSION

The experiments described in this paper demonstrate the existence of afferent fibres in the hypoglossal nerve of the cat. The fibres all enter the central nervous system in the bulbar region, but the exact pathway of the fibres into the cranium appears to vary. In certain cats all of the fibres apparently enter with the vagus-accessory group through the jugular foramen; while in other cats some at least of the fibres enter with the hypoglossal nerve through the hypoglossal canal. It is not possible to state whether, in the latter series of animals, afferent fibres enter with the vagus-accessory group as well, but this seems very probable. In the formation of the skull no rigid separation of the fibres of the three nerves occurs, and in the adult animal there is exchange of fibres between the nerves outside the skull (Sekita, 1931). The experimental findings recall the different arrangements of spinal nerve roots described by Langley (1900).

In the absence of more precise information as to the central origin of the fibres it is not possible to postulate the site of the sensory cell body. Certainly few, if any, of such cells are situated in the ganglion nodosum of the vagus, since removal of this ganglion does not appreciably affect the responses; but they do appear to lie in some relation to the vagus nerve inside the skull. No estimate can be made of the functional importance of Froriep's ganglion, described by Langworthy as being constantly present in the cat.

The results of section of the whole vagus-accessory-hypoglossal group close to the skull are of interest. Bearing in mind the previous reservations, they show that the afferent fibres have no connexion with the cervical spinal cord, in contrast to the findings of Sherrington, Langworthy and Corbin *et al.* No afferent fibres connect the tongue and spinal cord *via* the ansa hypoglossi, as found by van der Sprenkel. It has also been shown that the afferent fibres of the descendens cervicalis pass through the first cervical dorsal root, an observation which is in keeping with the anatomical origin of the nerve; whereas the observations of the previous authors indicate a connexion between the second cervical ganglion and the hypoglossal nerve. Further work must decide whether these contradictions are, in fact, due to species variations.

Since the hypoglossal, descendens hypoglossi, and descendens cervicalis nerves were stimulated close to their entry into muscle substance, it is probable that the end-organs of the afferent fibres lie in close relation to these muscles. Attempted stimulation of the end-organs in the tongue was ineffective and recalls the negative results of Barron and of Corbin & Harrison. Although the reflex responses used as indices may indicate only the receipt of impulses which are associated with a particular central interpretation, it appears reasonable to assume that impulses set up by stimulation of the end-organs should produce these reflex responses provided that an adequate number of such end-organs discharge. It is concluded that the absence of reflex responses to stimulation of the tongue is to be associated with the use

of inadequate stimuli. On the basis of the present evidence it is not possible to assign any specific role to these afferent fibres.

SUMMARY

1. Using pupil dilatation and rise of blood pressure as reflex indices of afferent impulses, afferent fibres have been demonstrated in the hypoglossal, descendens hypoglossi and descendens cervicalis nerves of the cat.

2. There is evidence that the afferent fibres in the stem of the hypoglossal nerve enter the medulla either with the vagus-accessory nerves or with the hypoglossal nerve also. Those of n. descendens cervicalis enter the spinal cord through the first cervical nerve.

3. Destructive stimulation of the tongue, with chorda-lingual and glossopharyngeal nerves cut, produced no reflex responses.

I am deeply indebted to Prof. B. A. McSwiney for his advice and encouragement. The expenses of this investigation have been defrayed in part by a grant from the Government Grant Committee of the Royal Society to B. A. McS.

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THE ORIGIN, DEVELOPMENT AND FUNCTION OF THE BLOOD CELLS IN CERTAIN MARINE TELEOSTS. PART I. MORPHOLOGY

By E. S. DUTHIE

From the Sir William Dunn School of Pathology, University of Oxford

Since Ehrlich in 1878 classified the granular leucocytes of mammals on the basis of their staining reactions, many attempts have been made to apply this classification to the whole vertebrate kingdom. Ehrlich's classification was justified by the mass of physiological data which soon became available and which established the specific nature of the neutrophil and the acidophil leucocytes. The function of the basophil leucocyte still remains unknown. In general, neutrophil leucocytes form the majority of the circulating granulocytes: they are actively motile and phagocytic, and play an active part in all acute inflammatory reactions. Their granules are finer than those of the acidophil or basophil granulocytes—an important point, since their tinctorial properties vary from species to species. Thus, in the guinea pig they are amphophil, in the rabbit acidophil, and in the cat, rat and mouse no granules can be demonstrated by ordinary stains. In the human they stain lightly though not specifically with most methylene-blue eosin combinations. Non-granular leucocytes (lymphocytes and mononuclears) are present in all mammals examined in about the same proportion, and with little morphological difference.

In birds the most recent authors, Forkner (1929) and Shaw (1933), found circulating leucocytes similar in morphology to those of mammals, and occurring in about the same proportions. In the pigeon (Shaw, 1933), the neutrophil cell is a pseudo-eosinophil with rod-shaped granules. In amphibia the mammalian types of leucocyte are still found, though there is little information in the literature regarding their numbers and proportions. A nucleated small cell, the thrombocyte, is found to be the homologue of the non-nucleated platelet of mammals (Jordan & Flippin, 1913; Jordan, 1919, 1925, 1938; Jordan & Speidel, 1923, 1924*a*, *b*).

Finally, in fishes there appears to be some difficulty in applying the usual leucocyte classification. Drzewina (1905, 1911) describes both fine and coarse granulocytes in a number of species examined. The former were definitely neutrophil and the latter usually acidophil. In certain fishes, e.g. *Acipenser sturo*, the coarse granulocytes were subject to great variation—acidophil, basophil, and hybrid forms occurring in the kidney tissue. He mentions a mononuclear cell in the blood of certain fishes, but does not describe it. Later, Jordan & Speidel (1924*a*, *b*) found neither mononuclears nor basophil granulo-

cytes, though lymphocytes, coarse acidophils and fine pseudo-eosinophils (neutrophils) were present. The coarse acidophil granulocytes have been described by various authors (Loewenthal, 1909; Lanine, 1912; Drury, 1914). Other authors (Gulland, 1898; Greene, 1912; Michels, 1923; and Bolton, 1933), have described the occurrence of large numbers of coarse basophil granulocytes in fishes, but did not find any acidophil forms. The present study was prompted by some chance observations on the staining reaction of the acidophil cells in *Trigla corax*, which led to an investigation of the development of the granulocytes and of haemopoiesis in the Triglidae and other families. In addition a number of experimental studies were made, which will be made the subject of a later paper.

MATERIAL AND METHODS

The material consisted of specimens of the following fishes: *Crenilabrus pavo* and *Trigla corax* were collected at the Stazione Zoologica, Naples, in the beginning of 1935; in most cases they were freshly caught and were not in the sea-water tank for more than one or two days before being killed. Specimens of *T. hirundo*, *T. cuculus*, *Labrus bergylta* and *L. mixtus* were collected at the Laboratory of the Marine Biological Association of the United Kingdom, Plymouth, during a short stay in August 1935, and in addition some material was collected there later and forwarded to me. The supra-vital observations, together with the examination of stained smears, were made in the autumn of 1937 at the Marine Biological Station, Port Erin, Isle of Man, on specimens of *Labrus mixtus* and *Trigla hirundo*; the former were freshly caught, while the latter were tank specimens. These observations were completed on specimens of *Labrus bergylta*, *Crenilabrus melops*, and *Trigla lucerna*, examined at the Station Biologique, Roscoff, France, in June 1938. Members of other families were examined, but were not included in this study, either because they were not available in sufficient numbers, or because nothing of interest was found. In order to repeat the observations of Michels (1923), a few specimens of *Cyprinus carpo* (the carp)—purchased through a dealer—were examined.

All specimens were killed and examined or fixed immediately, as fish material autolyses rapidly at room temperature. In the majority of cases small pieces of gill, spleen, kidney, liver and portions of the alimentary tract at various levels were fixed 12–18 hr. in Zenker-formol. Pieces of the skin and mucous membranes including the mouth and operculum together with the underlying tissues were sometimes included. They were washed for 24 hr. in running water, dehydrated slowly, embedded in paraffin and sectioned at 4–6 μ. Staining in Giemsa's solution at room temperature for 12–18 hr. gave the best results as a routine, though I have also used other methods. For the blood-forming organs the most successful results were obtained by fixing in methyl alcohol or in absolute alcohol containing mercuric chloride. The smears were brought down to distilled water, and were stained overnight in Giemsa's solution, one part in fifty at about *p*H 7·4. Thionin in 70 % alcohol as used by

Michels (1923), gave good results, but all these preparations fade completely in about 3 or 4 days. Spreads of peritoneum or swim bladder and blood spreads stained very well by this method. Dried blood smears failed to give such good results.

For differential white cell counts and reticulum staining I have adopted the following method. After stunning the fish by a sharp blow on the head, the tail was quickly amputated and the blood allowed to flow into waxed watch glasses containing the following solution modified from Nicholson (1934), brilliant cresyl blue 0·1 g., sodium citrate 0·55 g., sodium chloride 0·5 g., formol 0·1 c.c., distilled water 50 c.c. Mixing was carried out with a waxed pipette after which a drop was transferred to a cover slip, picked up on a clean slide, ringed with vaseline and examined. Spreads were made at the same time, and were fixed wet in methyl alcohol and stained in Giemsa's stain. Living blood preparations were made with perfectly clean cover-slips and slides, and were ringed in the usual way with vaseline. For the peroxidase and oxidase (phenolase) tests, I used smears fixed in formol 1 part, alcohol 9 parts, and counterstained in neutral red. The oxidase was the usual indol phenol blue test (Graeff, 1916). In the peroxidase test a little benzidene was ground freshly in water, filtered, and four drops of 3 % hydrogen peroxide added to 200 c.c Smears were brought to water and left in this solution until the granules stained. Acidification with a drop of glacial acetic acid gave a good blue colour to the granules (Lison, 1936). With one exception, *Trigla lucerna*, there were no species distinctions apart from size in the various blood and tissue cells examined. In my figures I have given the species from which the drawings were made, but my conclusions are applicable to all members of each family that I have examined.

MORPHOLOGY AND DISTRIBUTION OF THE CIRCULATING BLOOD CELLS

Leucocytes. Their number varied considerably, depending on any injuries the fish might have received. In normal specimens of both *Labrus* and *Trigla* there are about 30,000–40,000 per c.mm., including the so-called thrombocytes. They can be classified as follows, coarse granulocytes, fine granulocytes, lymphocytes and thrombocytes. A few primitive cells are occasionally present in the blood.

Coarse granulocytes. These are in all cases of the type described by Drzewina (1905, 1911), Michels (1923), and Bolton (1933), and are usually about 400 per c.mm. in the normal fish (Fig. 2, *r*, *t*).[1] They measure about 9·5 μ in diameter in the Labridae, though they are considerably larger when moving. They are actively motile (10 μ in 17 sec.), and for this reason are very irregular in shape, but they are not phagocytic. A round or oval nucleus with well-marked chromatin network lies at one pole, farthest from the advancing edge. The granules are large and numerous and easily laked by any injury. In the Labridae they are

[1] Figs. 2–7 comprise Plates I and II.

everywhere of the acidophil type, staining in various shades of red with Giemsa's stain or any other methylene blue-eosin combination, and red with Dominici's tri-acid stain. In this family they can be well fixed by ethyl or methyl alcohol in blood films or smears and resist previous drying. In the tissues of the Triglidae they are definitely basophil, with the exception of the skin and mucous membranes of the mouth, oesophagus, operculum and gills where they become acidophil in the superficial layers. Circulating leucocytes are basophil in the Triglidae. The basophilic granules in the Triglidae are not fixed by alcohol alone, so that Leishman stained films show a vacuolated cell. When mercuric chloride is added to the alcohol perfect fixation is obtained in blood films and smears. In both families they are well preserved in paraffin sections fixed in Zenker-formol. In one species *T. lucerna*, taken at Roscoff, the circulating granulocytes failed to stain with a basic or acid dye, but in all other species a definite blue (orthochromatic) or purple (metachromatic) colour was obtained with the usual basic dyes, when mercuric chloride was included in the fixative. In both families the granules of the coarse granulocytes fail to give the oxidase or the peroxidase reaction.

Fine granulocytes. These number some 8–12 % of the circulating leucocytes or about 4000–5000 per c.mm. in both families. In the living condition they measure about 8–9 μ in diameter. Their granules are much smaller than those of the coarse granulocytes, and while they are easily seen in the living cell, they are stained with difficulty by any of the usual methods. Fixed in methyl alcohol and stained with Giemsa's stain or thionin, they are sometimes bright red in kidney smears or spreads of mesentery (Fig. 2, *m*). More usually they are colourless (Fig. 2, *w*). The nucleus is large and stains faintly, it lies at one side of the cell and is often bilobed or heavily indented. In cresyl violet wet preparations the cell is easily recognizable by its densely stained cytoplasm in which the granules can be seen (Fig. 2, *v*). In dried blood films the granules disappear, the cytoplasm stains a light blue colour and the characteristics of the cell are lost. In living preparations of blood made according to the method of McCutcheon (1923), these cells are actively motile (10 μ in 30 sec.), and are the first and most constant in this respect. In these preparations one finds them adherent to the under surface of the cover-slip, and only visible by the movement of the highly refractile granules. They are much stickier than any of the other blood cells, and readily adhere to one another and to foreign particulate matter. Experiments in which Indian ink was mixed and shaken with the blood showed that they are actively phagocytic, and they form the majority of the cells in exudates. In both kidney and blood smears they and their precursors give positive peroxidase and oxidase tests in both cytoplasm and granules.

Lymphocytes and thrombocytes. Under this heading I have included all those circulating leucocytes which contain no granules and which show little motility. In size they vary between 2 and 5 μ, and they number between 25,000–30,000 per c.mm. In fixed and stained smears it is difficult if not

impossible to separate lymphocytes from thrombocytes, but in cresyl violet wet preparations the cells (Fig. 2, *s*), regarded as thrombocytes by other authors (Jordan, 1919, 1925; Jordan & Speidel, 1924*a*, *b*), are easily distinguished from the lymphocytes by their oval or pointed appearance. The difference between thrombocytes and undoubted lymphocytes (Fig. 2, *n*), is not always clear, especially as both forms change their shape readily in living preparations, and one can find many transition forms between the two. This makes differential counting difficult as Forkner (1929) found in the fowl, but about 30 % of these small cells appear to be lymphocytes. In calling the pointed and oval forms thrombocytes, I am merely adhering to the terminology of previous authors, since in clotting all these small cells adhere together, becoming centres from which the fibrin strands radiate. The large lymphocytes show some motility. None of these cells gives a positive oxidase or peroxidase reaction.

Erythrocytes. These number some 2,000,000 per c.mm. in both families. They measure about 8 by 2·5 μ in the Triglidae and 7 by 6 μ or larger in the Labridae. The majority show a reticular network in cresyl blue preparations (Fig. 2, *c*). With maturation the cells become more oval, the nucleus more hyperchromatic, and the reticular network is finally lost (Fig. 2, *d*). In dried and stained preparations one can distinguish small rounded cells—normoblasts, and in addition large distended cells with palely staining nucleus, which are degenerating adult cells (Jordan & Flippin, 1913).

ORIGIN AND DEVELOPMENT OF THE BLOOD CELLS

The main centre of blood-cell formation is the kidney (Fig. 5), where both erythrocytes and leucocytes develop side by side between the kidney tubules. Branching reticular fibrils which commence in the wall of the blood vessels extend between these developing cells, dividing them into small groups. They are beautifully shown by Wilder's (1935) modification of Bielschowsky's technique. Immature cells of both types are present in the splenic pulp, while the lymphoid centres (Drzewina, 1905), in the submucous coat of the intestine and in the mesentery are mainly leucocytic centres (Figs. 3, 7). Both types of development are most easily studied in kidney smears, fixed wet in absolute methyl alcohol and stained with Giemsa's stain or alcoholic thionin. The developmental stages are easiest to follow in the Labridae, and Fig. 2, which is drawn from such smears, is an attempt to arrange the cells along the lines of most probable development. Careful comparison of such smears using various techniques showed no fundamental difference between the smallest non-granular leucocytes found in the kidney (Fig. 2, *a*), and those present in the circulating blood (Fig. 2, *n*). Jordan & Speidel (1923, 1924*a*, *b*) use the term lymphoid haemoblasts for these cells, and since one can trace transition forms between these and the granulocytes and erythrocytes, the name would appear to be justified. Karyokinetic figures are frequently found in these cells,

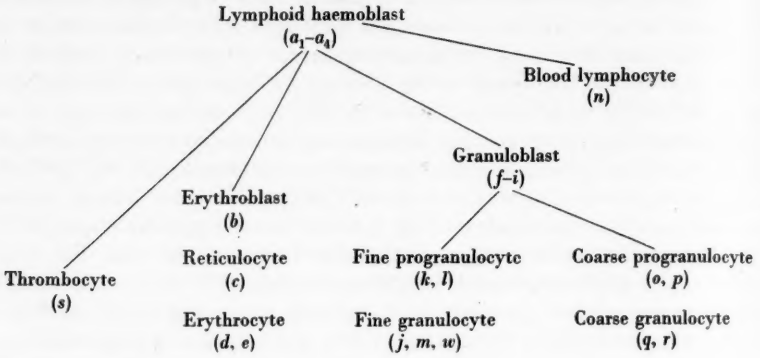
particularly the smaller ones. In sections they are often seen against the reticular fibres of the blood sinuses and the walls of the kidney tubules (Fig. 5, *LH*). These lymphocytes develop into granuloblasts (Fig. 2, *f–i*), which are larger and have a cytoplasm staining a deep blue with Giemsa's stain. In peroxidase stained smears no granules whatever are present in their cytoplasm, and division figures are to be found in the larger forms, showing that the smaller cells such as those shown in Fig. 2, *f*, are formed from them. Granuloblasts may develop either fine or coarse granules in their cytoplasm, at which stage I have termed them progranulocytes (Fig. 2, *k*, *l*, *o*; Fig. 3, *CPG*).

Fine progranulocytes (Fig. 2, *k*, *l*), are indistinguishable in Giemsa stained preparations from granuloblasts (Fig. 2, *f*, *g*), since the granules do not show, but in peroxidase stained preparations the differentiation is easy. The cytoplasm of the adult cell stained with Giemsa's stain (Fig. 2, *j*, *w*), is usually lightly stained granules good differential staining, owing to the large number of a pale blue colour with it contains. Under specially favourable conditions, particularly in thick smears, the granules may stain a reddish colour as in Fig. 2, *m*.

In the maturation of the coarse chromophil granulocytes there are several points of interest, which are illustrated in Fig. 2, *o–r*, and by the sections of mesentery (Figs. 3 and 7). The earliest progranulocyte differs only from the granuloblasts by the possession of a few coarse basophil granules in the cytoplasm. In the Labridae a large number of these are usually developed before the granule colour changes to brick red (Fig. 2, *q*) and finally to scarlet (Fig. 2, *r*), but occasionally in large progranulocytes (Fig. 5, *CPG*), a few acidophil granules may be present from an early stage. As in secretory cells of glands, the early granules form around the nucleus, and these newly formed granules are at first deeply basophilic. A comparison with the same stages in the Triglidae (Fig. 7), shows that in the latter the fully formed granules do not become acidophil in the blood-forming tissues, but enter the circulation while still basophil. Apart from staining reaction the granulocytes in the two families are identical in mode of origin, appearance and distribution. Division figures (Fig. 2, *u*), are sometimes found in these coarse granulocytes in the developmental centres.

Erythrocytes. The development from small lymphocyte to erythrocyte is most difficult to follow, though I feel sure that it occurs. The earliest erythrocyte stage that one can recognize is characterized by having a more hyperchromatic purple staining nucleus and a slightly acidophil cytoplasm (Fig. 2, *b*). I think that there exists a maturation division between the lymphocyte (Fig. 2, a_4), and the earliest recognizable erythrocyte (Fig. 2, *b*) and that the nucleus never goes out of the telophase, thus giving the characteristic hyperchromatic nucleus. At any rate there are no intermediate stages to be found, and cells identical with Fig. 2, *b*, are easily found in all stages of division. Further stages consist in the loss of the reticular material (Fig. 2, *c*, *d*), and the assumption of oval shape. The earliest cells such as those in Fig. 2, *b*, are never

present in the blood stream. The following developmental diagram will make the above conclusions clearer.



DISTRIBUTION AND SUBSEQUENT HISTORY OF THE GRANULOCYTES

Apart from the kidneys the largest collections of granular leucocytes are in the lymphoid centres of the mesentery and wall of the intestine. In the latter, apart from definite lymphoid centres they often occur in large numbers (Fig. 6), emigrating on to the surface of the mucous membrane. The same phenomenon is seen to some degree on all epithelial surfaces, and in the mucous membranes covering the gills and lining the opercular cavity, large collections of leucocytes lie close to the surface. In certain regions in the Triglidae, namely the external epithelium and mucous membranes of the mouth, oesophagus, gills, and opercular cavity, the staining reaction of the granules varies from basophil in the deeper layers to acidophil as the cells reach the surface. The change in staining reaction is abrupt from granule to granule, so that hybrid cells (Fig. 2, *x*) are fairly common in such regions as the gill-tip. Fig. 4 shows a portion of a gill filament in *Trigla corax* near its tip. Cells in the deeper layers are invariably basophil, but as they approach the surface epithelium (*SE*), the granules give an acidophil reaction. This was a constant phenomenon in all Triglidae examined, though the depth to which the acidophil leucocytes extended varied from fish to fish. In small fish, less than 150 g., they were usually not present as acidophils. The change has been verified by the repeated staining of sections taken from different animals, and is independent of the fixative and stain employed. With iron-alum haematoxylin no change could be distinguished.

In both Labridae and Triglidae I have found a peculiar alteration in the appearance of the granulocytes in their passage through the mucous membrane of the intestinal wall. This is best seen in Fig. 6, which is a section of a villus in the lower part of the intestine of *Trigla corax*. The granules in the migrating

cells stain more faintly (as in B), and then become more elongated and appear as pink acidophilic threads in the cytoplasm (C). At the same time the nucleus becomes hyperchromatic and the cell is orientated, so that the threads point to the surface of the mucous membrane. I have found this appearance repeatedly in teased preparations of living cells in all regions of the intestinal tract from the pyloric end of the stomach to the rectum, and at first I thought they were ciliated cells unrelated to the granulocytes. The distribution, size, and general morphology of the cells soon convinced me that they were derived from granulocytes, though I have never been able to produce such changes in

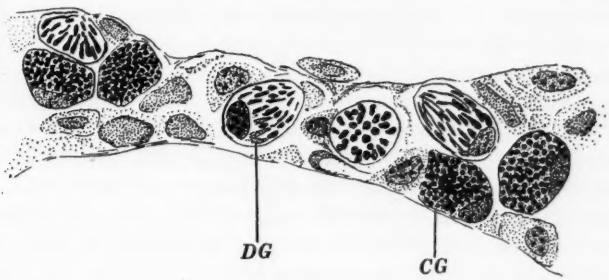


Fig. 1. Selection of the mesentery in *Labrus mixtus* showing maturing coarse granulocytes *CG*. Note discharged cells *DG* with elongated granules pointed towards the peritoneal surface. Zenker-formol Giemsa. × 1600.

the granules of the latter by physical or chemical means. I can only conclude that this is a definite functional phenomenon possibly determined by the age of the cell, and almost certainly representing a discharge of the granules on to the surface of the epithelium. This is borne out to some extent by the fact that a similar appearance is found only on the surface of the mesentery, i.e. in cells which have been discharged into the peritoneal cavity (Fig. 1), and occasionally in the kidney tubules into which coarse granulocytes may penetrate. Repeated examination of fixed material has never shown cells of this type among the granulocytes which are not in contact with an epithelial surface.

DISCUSSION

The majority of earlier workers on the blood cells of fish have been concerned mainly with the coarse granulocytes. Michels (1923) and Bolton (1933) have summarized most of the references. Of special interest to the present study are the following. Gulland (1898) noted the presence of large numbers of eosinophil granulocytes in the stomach wall of the Atlantic salmon (*Salmo salar*). Drzewina (1905) found granular leucocytes in the lymphatic tissue of various fish and amphibia. Under the heading lymphatic tissue were included those formative centres for the blood and lymph elements to be found between the tubules of the kidney, in the submucous coat of the intestine, in the connective

tissue of the liver, pancreas, mesentery, and genitalia, on the surface of the heart, and in the cranial cavity. In the Selachians *Raja clavata* and *Galeus clavus*, he distinguished clearly between coarse acidophil and fine acidophil granulocytes, but in *Labrus bergylta* he found only the former type. In the lymphatic tissue of *Acipenser sturo* a more marked degree of variation occurred. When the kidney and cranial lymphoid tissue was stained with magenta (basic fuchsin) or safranin and light green, the cells contained either acidophil or basophil granules or a mixture of both. With Unna's blue the granules were blue, green, or uncoloured. In the lymphatic tissue of the liver and heart the cell granules were all acidophil with Dominici's tri-acid stain, but with magenta or Benda's safranin and light green they were purely basophil. Drzewina considered these cells to be in various stages of development, and therefore to contain granules (amphophil) the chromaticity of which was uncertain. Later in 1911 he made a survey of the blood granulocytes of sixty-eight species of fish, and distinguished clearly in both selachians and teleosteans coarse and fine granulocytes. In the teleosteans he called the latter neutrophils, since the granules gave a grey violet colour with Ehrlich's tri-acid stain. Those pictured for the Labridae are clearly identical with the fine granulocytes described in this paper. In addition he mentioned that mononuclears were present in *Labrus mixtus*, but the description is vague and it is likely that they were poorly stained fine granulocytes. Specimens of various species such as *L. bergylta* and *Crenilabrus melops*, all of which had high numbers of eosinophil granulocytes in their blood and tissues, were placed in sea water the salinity of which was gradually decreased, so that at the end of 10 days, they were in sea water of 50 % of the normal salinity. They were kept a further 7–10 days in this diluted sea water, and at the end of this time the blood and tissues were examined. Drzewina found a gradual diminution in the size of the eosinophil granules, which stained more feebly and finally disappeared, while the cytoplasm became more and more eosinophilic and finally clear. The nuclei were unchanged and showed no degenerative changes. Similar results were obtained by increasing the salinity of the water. He also noted that the granulocytes disappeared from the blood and alimentary tract of certain Labridae during fasting, but were maintained by feeding. In fish caught in the Mediterranean there were fewer leucocytes than in those of the same species caught in the Atlantic. Karyokinesis was found in the lymphoid centres among the granulocytes and lymphocytes. Amitosis was also found to occur. All sectioned material was fixed in Zenker-iodine and Lindsay's fluid.

Downey (1909) described large numbers of granulocytes in the anterior end of the kidney of *Polyodon*. It was found impossible to classify the cells with blood stains into acidophil, neutrophil, and basophil, all shades of staining reaction being found in the same cell. Downey regarded these kidney leucocytes as being definitely secretory, and traced a dissolution of the granules which he regarded as an exhaustion of the secretion, followed by degeneration and phagocytosis of the cell. Werzburg (1911) made a survey of the blood cells

in a large number of poikilothermous vertebrates, which included fourteen species of teleosts. In these fishes dried blood films were fixed in the May-Grünwald stain, which was followed by staining in Giemsa's stain. The fish were classified into those having two types of granulocyte, those with one type of granulocyte, those without granulocytes but having instead amphochromophil agranular cells, and finally the perch which had only lymphoid cells. Werzberg's paper illustrates the danger of relying on one type of fixation and staining, and above all of not examining the living cells. None of the teleosts examined, except perhaps *Cyprinus carpo*, comes within the scope of the present paper, but from observations I have made on some of these fishes and from his illustrations it is clear that he was dealing with cells the granules of which were neither preserved nor stained by the method employed. His conclusions are therefore of little value and will not be considered further.

Greene (1912) described large collections of eosinophil cells in the gut wall of the king salmon (*Oncorhynchus tschawytscha*).

An experimental study of the coarse granulocytes of the peritoneal fluid of a large number of teoleosts was made by Drury (1914). The cells were present in all forms examined, but the stability of the granules varied from species to species. Cells were collected by the injection of hirudin in isotonic saline (one in ten thousand) into the peritoneal cavity, and examined by staining the cells with eosin and methylene blue in hanging drop preparations. The hirudin stabilized the cells. The cell granules in *Trigla gurnardus* took up the eosin readily, but in *T. hirundo* it was impossible to stain them. Injections of broth cultures of bacilli into the peritoneal cavity lowered the proportion of the granular leucocytes to the other cells, and made the granules more unstable. Injections of round worm extract into the alimentary canal produced no change in the numbers of the granulocytes there, nor did the presence or absence of food.

Michels (1923) studied the mast cells of the blood and connective tissues in amphibia, reptiles, and fishes. In certain species of all three classes there were enormous numbers of these cells either in the blood or connective tissues or in the latter alone. Three species of fish were examined, the white fish *Leuciscus* sp. and the carp *Cyprinus carpo*, both of which showed enormous collections of basophils in the connective tissues with few or none in the blood stream, and the eel *Anguilla vulgaris* in which none were present anywhere. In fish he seemed to think that such haematogenous cells as were present were primarily connective tissue granulocytes which had passed through the capillary walls.

Jordan & Speidel (1924*a*) studied haemopoiesis in certain elasmobranchs and teleosts. They found that the reticular cells of the spleen and kidney developed into lymphocytes both large and small. The small lymphocyte developed into thrombocytes, the larger into erythrocytes, special leucocyte, and eosinophils. Small lymphocytes were replenished by the amitotic division of both large and small lymphocytes. At the same time small lymphocytes could grow into the larger ones. In the wall of the intestine eosinophil leuco-

cytes were found to be formed from lymphocytes, and these in turn differentiated from connective tissue cells. In addition they might arise directly from connective tissue cells. The granulocytes were found to emigrate into the epithelial lining of the intestine and to disintegrate there. The authors described a final bursting and disintegration of the granulocyte, characterized by liquefaction of the granules and swelling of the cells. Later (1924*b*) they described a similar developmental process in the spleen and liver capsule of the salamander *Diemyctylus viridiscens*, while in the skate, *Raja ocellata*, two types of eosinophil, finely and coarsely granular, were found to arise from lymphoid haemoblasts. Early stages in these granulocytes were characterized by unripe basophilic granules. The granulocytes figured are identical with those described in this paper. Jordan (1926) found homologous granulocytes in the gut wall of the larval and adult frog, and traced their development from connective tissue cells. Early stages possess blue orthobasophilic granules, and developed first into those having purple metachromatic granules, and later into those having red acidophilic granules. The change apparently occurred as the granulocytes moved towards the lumen of the gut, and hybrid forms were found. In their final form the cells were typical polymorpho-nuclear eosinophils, lying among the lining epithelial cells. Jordan points out that these connective tissue granulocytes are morphologically similar to those described by Michels (1923) in fish tissues, which stained metachromatically red with thionin and were interpreted by him as being mast cells. Jordan's material was fixed in Helly's fluid and was stained in Giemsa's stain.

More recently, Bolton (1933) has described the occurrence of large collections of mast cells in the mucous membrane of the intestinal tract of the sock-eye salmon (*Oncorhynchus nerka*) and other salmonoid fish. The cells formed huge accumulations in the submucous coat, and were present between the crypts of the stomach glands. They were found at all ages from the 20 mm. fry to the adult fish, and at all times and in all parts of the alimentary tract, while their number bore no relation to the functional state of the tract. In the Pacific herring (*Clupea palasso*), he noted the occurrence of both eosinophil and basophil granulocytes, together with hybrid forms in which both types of granules were present. No suggestion could be made as to the function of the granulocytes. His material was fixed in Helly's or Maximow's Zenker-formol, and was stained in a neutral stain composed of ethyl-violet and Biebrich scarlet.

It will be seen from this brief summary that there are many points of difference between the conclusions of different authors. Because of their conspicuousness the coarsely granular leucocytes have been more often described, and apart from the papers of Drzewina (1905, 1911) and Jordan & Speidel (1924*a*, *b*) there is no mention of the finely granular forms. It is clear, however, that it is they which correspond morphologically and functionally to the mammalian neutrophil. The exact staining of the granules does not appear to matter, since in the selachians (Drzewina, 1911) and certain teleosts (Jordan & Speidel, 1924), the granules are pseudo-eosinophils as they are in many other

vertebrates. The coarse acidophil and basophil granulocytes bear certain resemblances to the coarse granulocytes of the mammalia. They form about the same proportion of all the granular leucocytes in the circulation, i.e. about 10%. They are non-phagocytic and are not particularly active in acute inflammatory conditions. In this connexion phagocytosis by mammalian eosinophils has been described. More comparable is their presence in large numbers in the submucous tissue of the intestinal tract like the mammalian eosinophil, and their presence just outside the wall of blood vessels in connective tissue, especially peritoneum, like the mammalian basophil. Their relation to allergic conditions should be of interest, if such can be evoked in fishes. In regard to the peroxidase and oxidase reaction they differ, since all three mammalian leucocytes give a positive reaction, whereas in fishes only the finely granular forms do so. In the case of these reactions varying results have been obtained by different authors, and it is far from certain what the tests employed signify (Lison, 1936). Shaw (1933) has examined the peroxidase reaction of the leucocytes of certain vertebrates. In man, rabbit, frog, owl and pigeon eosinophils were positive and basophils were negative. Polymorphonuclear neutrophils (pseudo-eosinophils) were positive in man, rabbit and the frog, and negative in the owl and pigeon. Shaw's statement that the basophils of mammalia are necessarily peroxidase and oxidase negative needs some qualification, since in the mesentery of the rat they give a strong positive reaction. In fish I have occasionally doubted the specificity of the reaction, since in a few cases which I was unable to repeat, I was able to obtain positive results with the coarse granulocytes and negative results with the fine, the opposite of what was usually found. For this reason one cannot rely on results obtained unless a standard technique has been followed, and unless a sufficient number of observations are made.

The other fish leucocytes, lymphocytes and thrombocytes are exactly as described by Jordan & Speidel (1924*a*), and are similar to those described in amphibian blood. They all appear to play some part in the clotting process. Mononuclears mentioned by Drzewina (1911) do not appear to exist in fishes.

The question of the staining reaction of the coarse granulocytes has led to considerable confusion in the literature. Drzewina (1905), Downey (1909), Jordan (1926) and Bolton (1933), noted differences in the staining reaction of the granules. Drzewina and Downey found differences in granules in the same cell in the blood-forming tissues. There is little doubt that these authors were dealing with immature cells, and there is no reason for grouping the cells as Downey suggests. I have found nothing in the nature of a dissolution of the cell granules in the kidney as described by Downey (1909) in *Polyodon*, and from his figures it would seem that many of the cells were coarse and fine granulocytes undergoing maturation, so that the term "secretory leucocyte" is confusing. Downey's material was fixed in formalin, which may be insufficient for fixing the cell granules he was dealing with. The cells he calls mononuclears correspond to granuloblasts and progranulocytes in my termin-

ology, and he quite correctly traces their origin from the lymphocytes and their development into granulocytes. In Figs. 54 and 55 of his paper, he figures binucleate granuloblasts of the type shown in Fig. 2, *h*, *i* of the present paper. Downey interprets the small nucleus of this binucleate cell as an ingested lymphocyte nucleus, an interesting possibility.

Michels (1923) found what he termed secretory leucocytes in large numbers in the digestive tract of the carp, and was under the impression that they differed from the blood leucocytes of the fish. For this reason I obtained some carp and investigated the character and distribution of their granulocytes. Michels used Maximow's (1913) technique of fixation in 100% alcohol, and staining in a saturated solution of thionin in 80% alcohol. Michels relied mainly on smears and spreads and in this way found large numbers of basophil granulocytes in the blood, mesentery, peritoneum, subcutaneous tissue and wall of the swim-bladder. With the digestive tract, muscle, liver, kidney, and gall-bladder he was less successful, since he submitted them to paraffin sectioning. Staining the sections with Dominici's stain, Delafield's haematoxylin, and alcoholic thionin, he found granulocytes the granules of which had disappeared, their places being taken by large vacuoles. These he concluded were secretory leucocytes differing from those found in spreads and smears. In repeating Michels's work I found similar changes in alcohol-fixed and paraffin-sectioned material. In spreads the coarse granulocytes of the various tissues were obviously alike and similar to those in the blood stream, being basophils. Their granules stained metachromatically with thionin as pictured by Michels, but weakly with the blue component of Giemsa's stain. Certain staining differences were noticed since the granules failed to retain any of the stains well, and one often found non-stained cells in the spreads and smears. Fixed in Zenker-formol and sectioned in paraffin the granules were well preserved, but failed to stain characteristically with an acid or basic stain. On the basis of their staining reaction with thionin in alcohol-fixed wet smears and spreads, I can only classify them as basophil cells. There is no need to suppose that Michels was using an imperfect specimen of thionin as suggested by Jordan (1926). The granulocytes of the intestine of the carp are thus identical with those found in the blood and other tissues. Jordan & Speidel (1924*a*) found what they called secretory granulocytes in the kidneys of the teleosts they examined. From their illustrations (Figs. 15, 22) they appear to be the fine granulocytes of the fish, and the so-called macrophage in Fig. 16 of their paper, with an ingested erythrocyte, is in my opinion a similar cell. The whole conception of a special secretory leucocyte is an unfortunate one, since it has come to be applied to any granulocyte giving an unusual staining reaction, a not uncommon finding in the fishes.

The change in staining reaction of the granules noticed by various authors is a well-marked feature in their development in all vertebrates. Basophilia (orthochromatic staining) precedes metachromatic or acidophilic staining (Arnold, 1906; Kardos, 1911; Downey, 1914, 1915; Michels, 1923; and many

others). Jordan & Speidel (1924*a*, *b*) and Jordan (1926) showed that a progressive increase in the acidophilic quality of the granules was part of the ripening process. Considering granulocyte formation in the intestinal tract of the scup, where eosinophils are numerous and the granules in their earliest stages have never more than a bluish tinge, Jordan (1926) says that this is due to a rapid ripening of the granules, the unripe basophilic state being greatly abbreviated or omitted. On this view maturation of the coarse granulocytes would be rapid in the Labridae and slow in the Triglidae, but I do not think that one should ascribe much importance to a colour change in the granules since it is probably influenced by physico-chemical factors in the surrounding tissues. Drzewina's (1911) experiments are of interest. Minor variations in staining reaction between granulocytes in different tissues in the same animal were common. Basophils showed all stages from blue to purple, and acidophils varied from brick red to scarlet in different organs in the same animal, but in any given tissue the staining was homogeneous throughout its substance. The change described in the staining reaction of the coarse granulocytes on certain epithelial surfaces may depend on some change in the surrounding medium. A number of experiments made by me to test this point were unsuccessful. Drury (1914) found that only in *Trigla gurnardus* were the coarse granulocytes of the peritoneal cavity acidophilic. This may have been due to his technique, but it is also possible that the cells are subject to variation in this region.

Regarding the origin of the blood cells, I am in agreement with the findings of Jordan (1919, 1925), Jordan & Speidel (1924*a*, *b*), in almost all respects. These authors (1924*a*) state that coarse granulocytes may arise from connective tissue cells. I have been unable to verify this point in spread preparations, although I have sometimes seen appearances which suggested it in section. It appears that granulocytes of both types in connective tissue arise only from lymphoid cells brought by the blood stream. According to Jordan & Speidel amitotic division is common in blood-forming cells. I have repeatedly found all stages of karyokinesis in practically all the cells drawn in Fig. 2, but at the same time many cells in apparent amitosis are to be found. The karyokinetic figure in the adult granulocyte (Fig. 2, *u*) is of some interest. Michels (1923) found mitosis in adult mast cells only in the spleen of *Gongylus occelatus* (the lizard). Drzewina (1905), Downey (1909) and Bolton (1933) found no examples in fishes. The cell in Fig. 2, *u* was taken from an animal which had received an intraperitoneal injection of blood three days previously, and this had led to intense haemopoietic activity.

Finally, as regards function it would appear that the lymphocyte acts as a stem cell to all other forms, but may play some part in the clotting mechanism. The fine granulocyte carries out many of the functions of a mammalian neutrophil. Drzewina (1911) found that during starvation, the coarse granulocytes found in the blood and intestine were diminished in number, but the results were in some respects contradictory. Drury (1914) and Bolton (1933) did not find any difference in starvation, and the former concluded that bacteria alone

were an adequate stimulus. Drury showed that peptone on which staphylococci had been grown brought about early disintegration of the cell, and caused a breakdown in the granules. This is essentially what happens on a mucous or epithelial surface, and the discharge may have bactericidal properties. I am unable to explain the special form that the discharge takes in the intestine, peritoneum, and kidney, but it may be conditioned by the pressure of surrounding cells, compelling the granules to burst in one direction—outwards. On other surfaces there is no lack of disintegrating granulocytes with their granules clumped. Experimental studies should solve some of these problems.

SUMMARY

1. The circulating leucocytes of the Labridae and Triglidae consist of fine neutrophil granulocytes, coarse chromophil granulocytes, lymphocytes, and thrombocytes.

2. The fine neutrophil granulocyte is the homologue of the mammalian neutrophil, while the coarse chromophil granulocyte resembles in several respects the mammalian acidophil and basophil. Lymphocytes act as stem cells to the other leucocytes in connective tissues.

3. In the haemopoietic tissues the granular leucocytes are derived from pro-granulocytes, which in turn develop from agranular cells (granuloblasts). The primitive cell from which all the blood elements develop is a lymphoid haemoblast.

4. The coarse chromophil granulocytes are acidophil in the Labridae. In the Triglidae they are basophil except on certain epithelial surfaces where the granules become acidophil. In both families the cells disintegrate and the granules discharge on the main epithelial and endothelial surfaces, including the lining of the kidney tubules. It is believed that they serve to protect these surfaces.

5. The coarse granulocytes (acidophil or basophil) found in the tissues and blood of fishes are identical in all respects. The term secretory granulocyte as used by many authors implies a distinction which does not exist.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 2. Blood and tissue cells from *Labrus bergylta* and *Trigla corax* illustrating the development and morphology of the blood cells. *t* and *x* only from *T. corax*. *a, b, f, g, h, i, j, k, l, o, p, q, r* are from smears of kidney. *c, d, e, n, s, v, w* are from the blood. Alcohol-Giemsa indicates fixation in methyl alcohol of the wet smear followed by Giemsa's stain. With the exception of the paraffin sectioned cells *t, u, x*, all cells are drawn to the scale given with the figure. *t, u, x* are drawn to twice this magnification.

Fig. 3. Section of small lymphoid centre in mesentery of *L. mixtus* among pancreatic acini *AC*. *FS* indicates fat spaces. The small cells *L* are small lymphoid cells from which the granuloblasts and acidophil granulocytes develop.

Fig. 4. Portion of an epithelial surface near the tip of a gill filament of *T. corax*. Coarse basophil granulocytes are present in the subepithelial layers, but in the epithelium they are acidophil.

Plate II

Fig. 5. Section of haemopoietic intertubular tissue of the kidney in *L. bergylta*. Note lymphoid haemoblasts and coarse progranulocytes in various stages of development. Fine granulocytes can be distinguished by their large round or indented nuclei.

Fig. 6. Portion of the intestine of *T. cuculus*, showing large numbers of coarse basophil granulocytes in a villus, passing out between the epithelial cells. Discharged cells *C* are seen near the surface of the mucous membrane.

Fig. 7. Small lymphoid centres in the mesentery of *T. cuculus*. Note the numerous coarse granulocytes, in this case basophil, in various stages of development. One is seen in the centre of a small vein.

a_1–a_4	Lymphoid haemoblasts, Alcohol-Giemsa.
b, *d*, *e*	Stages in erythrocyte development, Alcohol-Giemsa.
c	Reticulocyte supravitally stained in cresyl violet.
f–*i*	Granuloblasts, Alcohol-Giemsa.
j	Adult fine neutrophil granulocyte, Alcohol-Giemsa.
k, *l*	Fine progranulocytes, fixation formol-alcohol peroxidase reaction.
m	Adult fine granulocytes in peritoneum, fixation methyl-alcohol stained Pappenheim's panoptic stain.
n	Lymphocytes, Alcohol-Giemsa.
o–*q*	Coarse acidophil pro-granulocytes, Alcohol-Giemsa.
r	Acidophil granulocyte.
s	Thrombocytes, cresyl violet supravital staining.
t	Coarse basophil granulocyte *T. cuculus* fixation Zenker-formol.
u	Coarse acidophil granulocyte from mesentery showing karyokinesis, Zenker-formol Giemsa.
v	Fine granulocyte, supravitally stained in cresyl violet.
w	Fine granulocyte in amoeboid movement, Alcohol-Giemsa.
x	Basophil granulocyte in transition to acidophil form in gill tip of *T. cuculus*, Zenker-formol Giemsa.
LH	Lymphoid haemoblasts.
FG	Fine granulocytes.
CG	Coarse granulocytes.
EC	Erythrocytes.
GB	Granuloblasts or fine progranulocyte (indistinguishable).
CPG	Coarse progranulocytes.
EB	Erythroblasts.

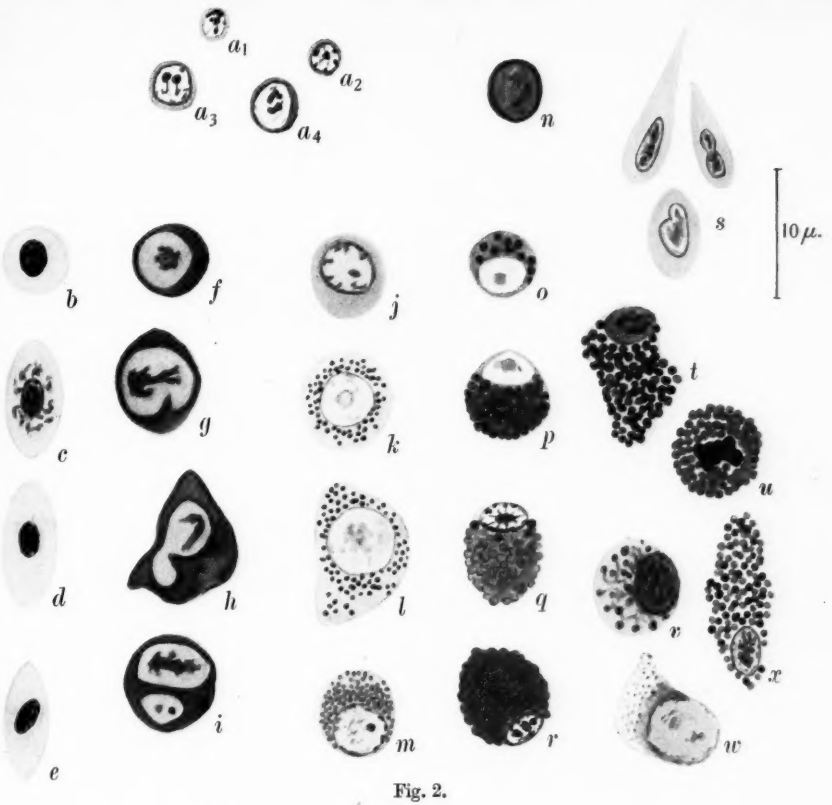

Fig. 2.

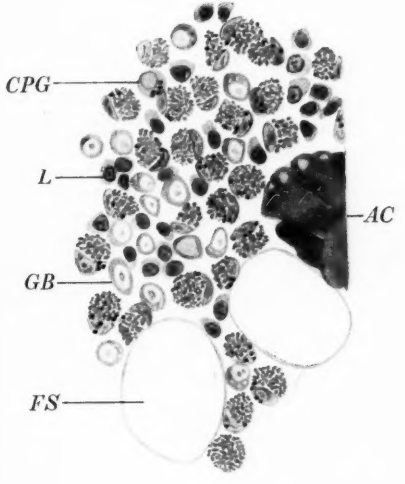

Fig. 3.

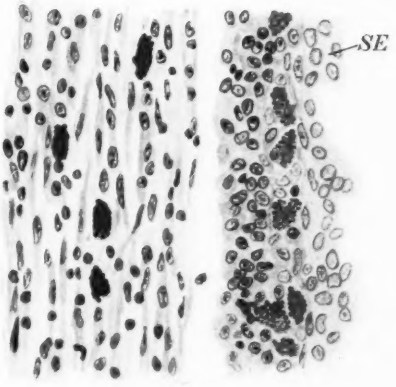

Fig. 4

10 μ.
50 μ.

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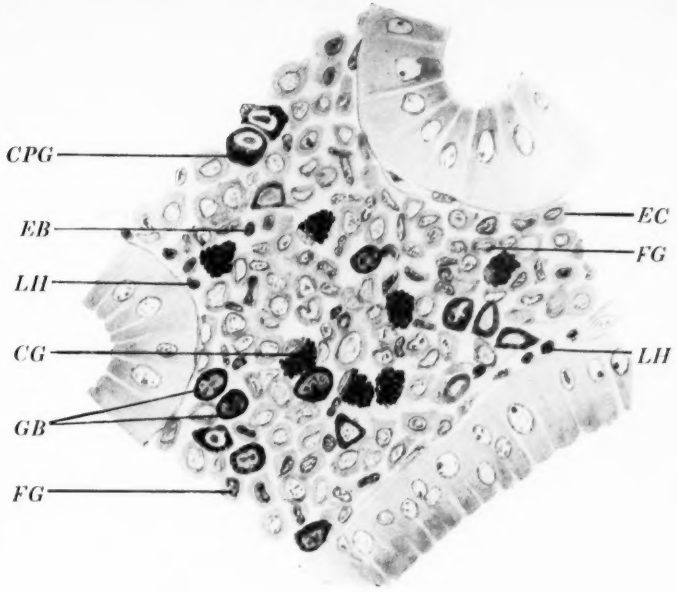

Fig. 5.

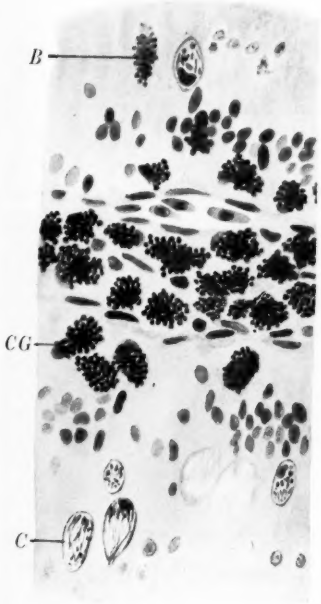

Fig. 6.

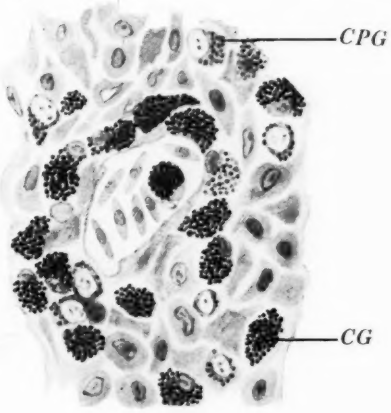

Fig. 7.

10 μ.
50 μ.

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THE HAEMOLYMPH NODES OF THE RAT (IRON PIGMENT LYMPH NODES)

By HANS SELYE and VICTOR SCHENKER

From the Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada

Numerous investigators have described haemolymph nodes in the rat (Vincent & Harrison, 1897; Drummond, 1900; White, 1904; Macmillan, 1928). They are usually small, flat structures measuring up to 3 mm. in diameter, and in some cases it is not easy to recognize them without the help of a microscope. Although Clarkson (1891) was unable to find them, our own observations on several hundred albino and "hooded" rats have convinced us that they are constant structures in the vicinity of the adrenals on both sides, situated dorsally to the adrenal artery and vein. They are also quite constant in the pancreas, and occasionally we have seen similar nodes in the vicinity of the thymus. Selye & Foglia (1938) emphasized that although these nodes are quite conspicuous by their dark brown colour, which distinguishes them from the ordinary lymph nodes, their sinuses rarely contain any noteworthy quantity of free blood. Usually the sinuses, especially the peripheral sinus, are filled with Russell-body cells, or erythrophages. Since these latter contain either more or less decomposed erythrocytes or haematogenous iron pigment deposits, they give a characteristic reddish brown colour to these structures. The absence of free circulating blood in the sinuses of these nodes makes it necessary to distinguish them from the true haemolymph nodes or haemal nodes such as have been described in other species. The term "iron pigment lymph nodes" has therefore been suggested.

Selye & Foglia (1938) found, furthermore, that such iron pigment lymph nodes may be produced experimentally in the rat following exposure to damaging agents capable of eliciting an "alarm reaction" (excessive muscular exercise, exposure to cold, and toxic doses of formaldehyde). Under the influence of such agents the ordinary lymph nodes of the rat may be transformed into iron pigment lymph nodes within a few hours. This is particularly obvious in animals whose blood volume has been increased by the intravenous administration of physiological NaCl solution in large quantities prior to exposure to the damaging agent. Under these conditions, the sinuses of the ordinary lymph nodes are first invaded by erythrocytes which are then engulfed by macrophages (erythrophages) and finally transformed into brown pigment granules which give a positive Prussian blue reaction.

It seemed rather interesting that although ordinary lymph nodes may be transformed into iron pigment lymph nodes in the rat, the latter are always

located in exactly the same position. The question arose whether the organ whose lymph they drain plays any part in causing these nodes to take up and destroy red cells. It seemed to us that in the case of the renal iron pigment lymph node, this question could be subjected to experimental investigation, for the only two organs whose lymph might possibly drain into these nodes are the adrenals and the kidneys. By removing one or both of these organs on one side, it should be possible to determine whether or not blood destruction in the renal lymph node is dependent on the presence of the adrenal or the kidney, or whether it is the result of an inherent peculiarity of this particular lymph node. Before describing our experiment, we wish to emphasize again that, contrary to the statements of most investigators who have examined haemolymph nodes of other species, we find that in the rat the sinuses of the iron pigment nodes are filled with lymph and must consequently be supplied by lymph vessels.

Inasmuch as immature rats usually possess no iron pigment lymph nodes, while adults regularly have them, we divided our experiments into two series, one of which was carried out on immature rats (6 weeks old), in order to see whether iron pigment lymph node formation could be prevented; the other was performed on adults (1 year old), to see whether such nodes, once formed, could revert to the normal lymph node type. In each of these series 24 rats were used. In six of these the left kidney was removed, in six others the left adrenal was extirpated, and in six both adrenal and kidney were removed on the left side. In all these cases special care was taken not to injure any of the adjacent tissues. For this purpose the nephrectomies were performed by the intracapsular method, and the adrenalectomies without removing much of the surrounding fat tissue. The six remaining animals were left as untreated controls.

All animals were killed 5 months after operation. At this time iron pigment lymph nodes were found in the renal region of all untreated controls. In the unilaterally adrenalectomized group, the iron pigment lymph node on the operated side was not significantly different from that on the right side either in the young or in the old animals, and there is no indication in our experiment that the adrenal plays any part in the formation or maintenance of these nodes. In the nephrectomized groups, on the other hand, the iron pigment lymph nodes on the operated side were small and relatively pale both in the young and in the old animals, while on the right side they showed particularly marked development and pigmentation, which might possibly be ascribed to the compensatory hypertrophy of the remaining kidney. In the group in which both the adrenal and the kidney were removed, the nodes on the operated side had the same colour as ordinary lymph nodes without any trace of pigmentation, while the contralateral lymph nodes were greatly enlarged and dark red.

Histological examination revealed no difference between the young and the old animals in any of the groups, but confirmed our macroscopical findings.

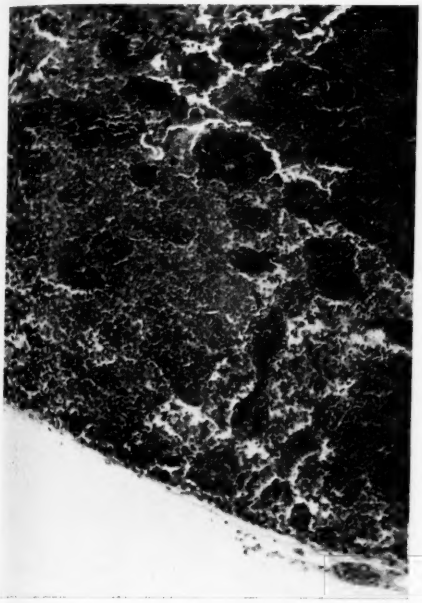

Fig. 2.

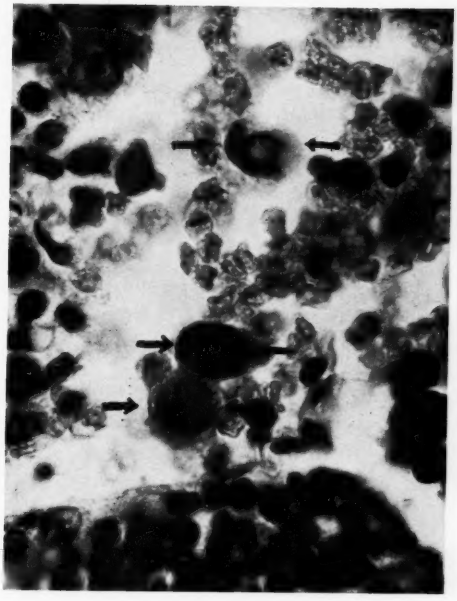

Fig. 4.

Fig. 1.

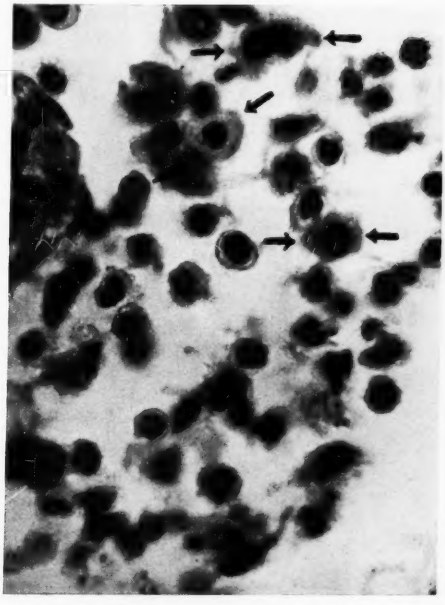

Fig. 3.

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Adrenalectomy did not seem to cause any noteworthy deviation from the normal; nephrectomy caused a marked reduction of pigment and complete disappearance of free or engulfed erythrocytes from the node on the side of the operation and increase of pigmentation with the appearance of numerous free erythrocytes and erythrophages in the contralateral node. In the case of combined nephrectomy and adrenalectomy, both pigment and red cells were entirely absent on the operated side in all but one case in which a moderate amount of pigment, but no red cells were observed. The contralateral lymph node on the other hand, contained numerous free erythrocytes, erythrophages and pigmented macrophages in its sinuses (see Pl. I, figs. 1–4).

It should be emphasized that although red cells (and their products of decomposition) disappeared entirely from the sinuses of the renal nodes following homolateral nephrectomy, especially when combined with adrenalectomy, these nodes still maintained a structure different from that of ordinary lymph nodes, in that they did not contain germinal centres.

SUMMARY AND CONCLUSION

Unilateral nephrectomy, especially when combined with adrenaléctomy, causes complete disappearance of erythrocytes from the sinuses of the renal lymph node in the adult rat. If these operations are performed in the immature rat before the transformation of the renal lymph node into an iron pigment lymph node, then they prevent the occurrence of such a transformation. It appears, therefore, that the blood-destroying function of the renal lymph node is dependent upon the presence of the kidney and possibly to a certain extent also upon the presence of the adrenal. In the absence of these glands, the node discontinues its blood-destroying activity but remains different from other lymph nodes in that it contains no germinal centres.

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EXPLANATION OF PLATE I

Fig. 1. Medium magnification of the peripheral portion of a left renal lymph node following removal of the left kidney and adrenal. Note that the sinuses contain only white blood cells and macrophages but no erythrocytes.

Fig. 2. Peripheral portion of the right renal lymph node following removal of the left kidney and adrenal. Same magnification as Fig. 1. Note the large number of erythrocytes in the sinuses.

Fig. 3. Portion of a sinus from the gland shown in Fig. 1. Oil immersion. No erythrocytes are seen but there are unpigmented macrophages (designated by arrows) and lymphocytes.

Fig. 4. Portion of a sinus from the gland shown in Fig. 2. Oil immersion. Erythrocytes are plentiful and three iron pigment-containing macrophages are seen (designated by arrows) free in the sinus.

A NOTE ON THE EFFECT OF COLCHICINE ON THE SKIN OF YOUNG RATS

By ALICE CARLETON, M.A. Oxon.

The question whether, in young animals, the growth of the skin and hair follicles (as evidenced by mitosis) shows any steady rate of progress, and whether, over the surface of the body, there are any areas which might be considered as focal points of growth, has been studied by the colchicine technique.

Eight rats were used. Rats 1, 2, and 3 were 6 weeks old and weighed about 60 g. each. They were given 0·03 mg. of colchicine subcutaneously, in a dilution of 5 mg. in 100 c.c. of distilled water. This is equivalent to a dose of 0·5 mg. per kg. of body weight. Three more rats, 1 day old and weighing 1·25 g. were each given a similarly proportioned dose, but died within a few hours. The dose was therefore reduced for their fellows of the same litter (4, 5, and 6), who received 0·0006 mg. Rats 7 and 8 were 12 days old. Rat 7 weighed 16·2 g. and rat 8, 16·7 g. They each received 0·006 mg. of colchicine in 0·4 c.c. of water.

As it has been shown (Carleton, 1934) that there is a diurnal rhythmical periodicity in mitotic division with a maximum between 8 p.m. and midnight, all the animals were given their injections at the same time, viz. midnight, and killed next day at 10 o'clock. Pieces of skin were then removed from the centre of the scalp, the middle of the back, above the umbilicus, above the symphysis pubis, the elbow, the antecubital fossa, the knee, the popliteal fossa, the dorsum of the fore paw, the palm, the dorsum of the hind paw, and the sole. It took about 15–20 min. to carry this out in each case, but the chances of mitoses completing themselves in this period were minimized by the colchicine. The pieces were fixed in Carnoy's fluid for 4 hr., transferred to absolute alcohol overnight, and cleared in cedarwood oil. After being embedded in paraffin, they were cut at 5 μ, and stained with haematoxylin and eosin. The cells of the epidermis and hair follicles were counted under the oil immersion. The percentage number is based on a count of 3000 cells in three different sections. All the stages from prophase to early daughter cells were regarded as mitotic. The counts were all made by one observer to preserve the same standard of judgement.

Butcher (1935) has shown that in albino rats there are cycles of hair growth, with a growing stage and a resting stage of about 17 days each. The first growth period commences on the first day, the second on the 34th, and the third on about the 70th day. It will be noted that the three groups of rats used, being respectively 1, 12 and 42 days old, are all in a stage of hair growth, and therefore from this aspect, similar pictures might be expected.

The results were as follows:

Table I

	Rats 6 weeks old, 60 g. 0·03 mg. colchicine (i.e. 0·5 mg. per kg.)			Rats 1 day old, 1·25 g. 0·0006 mg. colchicine (0·0008 had proved fatal to brothers)			Rats 12 days old 16·7 g. 0·006 mg. colchicine	
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
A. Top of scalp	0·5	1·0	1·0	0·7	2·0	1·8	22·4	2·0
B. Middle of back	0·5	1·9	0·2	1·4	1·3	2·2	21·2	2·3
C. Above umbilicus	0·2	0·3	1·2	1·4	2·0	1·9	19·6	1·8
D. Above pubic symphysis	0·3	7·1	0·8	1·6	1·3	5·6	20·3	1·8
E. Elbow	0·1	0·7	0·3	1·4	1·2	1·3	21·9	1·8
F. Antecubital fossa	0·5	0·7	0·4	1·3	1·6	1·2	21·2	1·4
G. Knee	0·8	4·2	6·8	1·5	1·2	7·7	20·3	1·8
H. Popliteal fossa	0·7	1·6	0·5	2·2	1·8	1·7	15·7	2·3
I. Dorsum of hand	0·3	0·8	0·1	1·8	2·0	1·5	2·7	0·4
J. Palm	0·4	0·8	0·7	1·5	1·5	2·0	5·4	0·8
K. Dorsum of foot	0·7	0·8	0·4	1·6	2·3	2·4	5·9	0·7
L. Sole	0·4	0·8	0·7	1·0	1·0	0·9	4·3	0·9
Average	0·45	1·7	1·1	1·4	1·6	2·5	15·0	1·5
		1·1			1·8			

It will be seen that, excluding rat 7, the average rate of growth for the three rats at 1 day old is 1·8 %. Rat 8 at 12 days old shows an average of 1·5, and the three rats at 6 weeks old an average of 1·1 %. There appears, therefore, to be a steady diminution in the rate of cell division from the first day onwards.

Table II

Average for	Including rat 7	Excluding rat 7
A. Top of scalp	3·9	1·3
B. Middle of back	3·9	1·4
C. Above umbilicus	3·5	1·2
D. Above pubic symphysis	4·6	2·6
E. Elbow	3·6	1·0
F. Antecubital fossa	3·5	1·0
G. Knee	5·4	3·4
H. Popliteal fossa	3·3	1·5
I. Dorsum of hand	1·2	1·0
J. Palm	1·5	1·1
K. Dorsum of foot	1·8	1·3
L. Sole	1·2	0·8

Again excluding rat 7, we note that, with the possible exception of D and G (the regions above the umbilicus and on the front of the knees respectively), the rate of growth does not vary very much over the surface of the body, having an average of 1·16 with extremes varying from 0·8 to 1·5. It has been shown (Carleton) that in nine young white mice the average percentage of mitoses in the skin of the abdomen at midnight was 1·4, with extremes of 1–2.

Of more importance is the observation of the extremely different effect of colchicine on animals of the same species and, in certain cases, of the same age. The same dose proportionate to the body weight produced no general rise of cell division in the first three rats, aged 6 weeks (using the mitotic rate in

normal young mice as a standard of comparison) though a local rise was noted in rat 2 D and G, and rat 3 G, but it caused death within a few hours in the next three treated, which were 1 day old. A slightly diminished dose in rats 4, 5, and 6, from the same litter as those who died, and rat 8, aged 12 days, caused a small rise, whereas in rat 7, from the same litter as 8, a remarkable increase in mitoses up to an average of 15 % appears. And yet rat 7 weighed somewhat less than rat 8 (16·2 as compared with 16·7 g.), and might therefore be expected to display less not greater evidence of cell division.

Since colchicine is likely to be widely used as a control in endocrinological experimental work, it seems important to draw attention to its uncertain effects. Allen *et al.* (1937), working on the effects of Theelin in ovariectomized mice, used eleven animals, with two controls treated by colchicine only, but judging from the observations in this paper, this number would appear to be insufficient. Further, one of the two controls was not killed till 24 hr. after the injection, whereas it is established that cells begin to recover from the effect of colchicine in 9–10 hr. An animal killed 24 hr. after injection is therefore valueless as a control. Until a more extensive knowledge of the range of individual variations in different species has been acquired, a large number of controls is necessary to eliminate gross error.

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ON THE RELATIONSHIP BETWEEN THE EPIPHYSIS CEREBRI AND THE REPRODUCTIVE SYSTEM

By SHAFIK ABD-EL-MALEK, M.B., B.Ch.
Anatomy Department, Medical Faculty, Cairo

From the Histological Laboratory, Physiology Department, Cairo

INTRODUCTION AND LITERATURE

The suggestion that some functional relationship exists between the pineal body and the genital organs finds its origin in clinical reports that tumours of this gland are at times accompanied by sexual precocity. As a result of clinical observations, Marburg (1907) formulated the idea that the pineal body during childhood inhibits both sexual and somatic growth, and that when this gland is destroyed by tumours, growth (sexual or somatic, or both) begins earlier and continues uncontrolled. Various authors have since endeavoured to prove such a relationship, but the results obtained are of a contradictory nature and the question still remains unsolved.

Engel (1935) thinks it possible to arrest the gonadotropic effect of the anterior lobe of the pituitary by administration of epiphyseal extracts. On repeating the same experiments in the rat and rabbit, Fleischmann & Goldhammer (1936) found that injection of epiphyseal extract, as well as subcutaneous implantations, have no effect on the action of gonadotropic hormones.

The present investigation arises as a result of still another contradictory finding. Fleischmann & Goldhammer claim to be able to arrest regular oestrus in rodents through the implantation of the epiphysis of infantile animals. They found that aseptic subcutaneous implantation of the epiphysis taken from two recently killed female rats (10–20 g. each) into an adult female mouse led to an arrest of its oestrous cycle in dioestrus. The period of arrest varied from 4 to 6 weeks, after which time the regular cycle reappeared. In some animals only one cycle was missed. Histologically the follicles became smaller, but no degeneration in the ovary was observed. The authors believe that for the success of the experiments, implantation must be made during the maximal development of oestrus, and that mice with an oestrous cycle of at least 5–8 days' duration are preferable. Tarkhan (1937) repeated the same work in six mice, precisely following the steps recommended by the previous authors. Only in one mouse did the cycle stop in dioestrus. He states that the presence in the epiphysis of infantile animals of a hormone antagonistic to sexual hormones must be still regarded as a matter open for enquiry.

It must further be stated that, so far, no substantial histological proof in favour of the glandular nature of the epiphysis has been brought forward. The contradictory reports made by the various investigators as a result of their experiments call for a continuation of the investigations of this problem.

MATERIAL AND METHODS

The following series of experiments were carried out in order to test the statements advanced by Fleischmann & Goldhammer.

I. In the first series of animals the experiments of Fleischmann & Goldhammer were repeated, following the same technique except that rats were used instead of mice. As a preliminary, the oestrous cycle was followed for a sufficiently long time in a group of twenty adult rats. Eight of them were

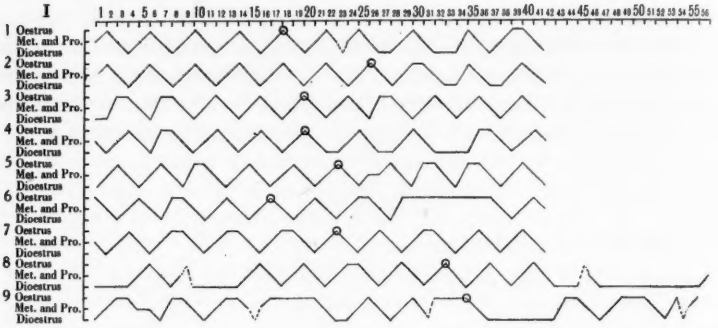

Fig. 1. Curves showing the course of the oestrous cycle in nine rats before and after implantation with a variable number of epiphyses of infantile rats. The circular mark on each curve indicates the date of implantation.

used as controls, three of which proved to have an irregular oestrous cycle. Control operations were performed on three other rats, but with entirely negative results (i.e. without effect on their cycle). The remaining nine were implanted with a variable number of pineal glands obtained from infantile rats weighing about 20 g. each.

As shown in Fig. 1, rats nos. 1 and 2 were each implanted with the epiphyses of four male infantile rats, no. 3 was implanted with five epiphyses also from male infantile rats. These three animals showed no change in their oestrous cycle. No. 4 was implanted with three epiphyses, one from a female and the two from male rats. The result obtained was the same as in the preceding animals. No. 5 was implanted with two epiphyses of female rats, and nos. 6 and 7 were each implanted with three epiphyses of female rats. No change in their oestrous cycle was observed.

Nos. 8 and 9 were respectively implanted with two and three epiphyses of female infantile rats. The implantation was followed by a cessation of the

oestrous cycle for 8 days in the first and for 6 days in the second animal. It is not possible, however, to accept these results as lending support to the claim of Fleischmann & Goldhammer, because the oestrous cycle of the two animals in contrast with the others of the same group presented definite irregularities, even in the pre-operative period. A comparison with control animals shows that similar short prolongations of the dioestrous stage are frequently encountered in perfectly normal animals.

II. A series of twenty-two rats were taken; their oestrous cycle was followed over a period of time and found to be regular. They were each implanted under aseptic precautions with a quarter or a half epiphysis of a calf 2–3 months old.

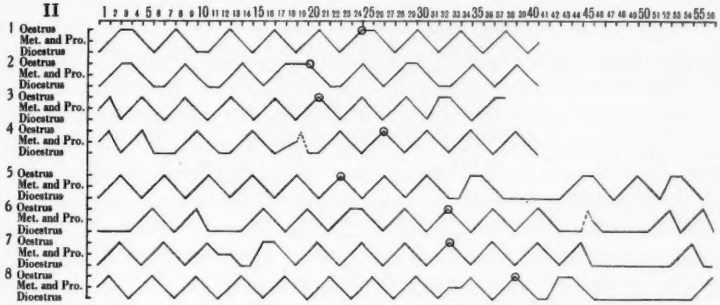

Fig. 2. Curves showing the oestrous cycle of eight rats implanted with calf's epiphysis. The first four curves represent practically normal cycles; the other four curves show a short prolongation of dioestrus, not immediately following the operation.

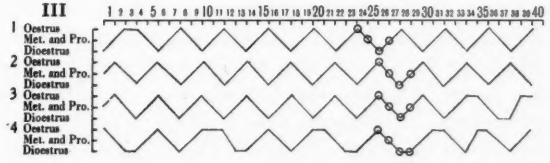

Fig. 3. Curves showing that the subcutaneous injections of epiphysan in the rat has no effect on its oestrous cycle.

The treatment with the epiphysis of the calf remained without any effect on the oestrous cycle in eighteen animals. In four animals there was a short prolongation of dioestrus which did not, however, immediately follow the operation but occurred after one to three normal cycles. Fig. 2 shows the curves of the cycle in these four experiments.

III. Eighteen rats in which the oestrous cycle was observed over a period of time were chosen. The period of their oestrous cycle varied from 4 to 5 days. These animals were injected with epiphysan "Richter", an extract previously used by other authors, and believed to contain the active principle of the

pineal gland. Each rat was given four ampoules of epiphysan on four consecutive days. Injections were always made at the peak of oestrus (each injection containing 0·10 g. of the fresh gland). In none of the animals was the oestrous cycle modified (see Fig. 3).

DISCUSSION

It is necessary to mention that our experiments differ in one respect from those of Fleischmann & Goldhammer. These authors implanted infantile epiphyses of rats into female mice, while in our experiments the infantile epiphyses of rats were implanted into animals of the same species. This was done because of the insufficient number of female mice at our disposal. However, we feel justified in regarding this difference as not essential; because, if the epiphysis of infantile rats does exercise any influence on the oestrous cycle of a mouse into which it is implanted, it should naturally exercise the same effect in an animal of its own species. We have implanted as many as five epiphyses with entirely negative results. The six animals in which a short arrest of the oestrous cycle took place showed no comparison with the observations of Fleischmann & Goldhammer in whose experiments the cycle usually stopped for periods of 4–6 weeks immediately following the implantations. In our own experiments, the prolongation of the stage of dioestrus did not in any case exceed that sometimes observed in perfectly normal animals, i.e. 6–8 days, and usually did not immediately follow the operation but occurred after a variable number of intervening normal cycles.

Fleischmann & Goldhammer emphasize that for the success of the experiments the implantation should be made at the peak of oestrus and preferably in animals in which the cycle is not less than 5–8 days. We have tried to comply with these conditions although we are of the opinion that they are of little or no significance. Indeed, if the implant is supposed to secrete a substance which arrests a cycle of 5–8 days for 4–6 weeks, why should the effect of such a potent substance be dependent on its being administered at the height of oestrus? The same consideration applies to the suggestion that it is necessary for these experiments to use animals with abnormally long cycles. If there were really an active substance secreted by the implant, it is difficult to understand why such a substance should act in animals with a cycle of more than 5 days and fail to do so in those with a normal cycle (i.e. about 4–5 days).

CONCLUSIONS

1. The experiments described in this communication fail to support the claim made by Fleischmann & Goldhammer that implantations of infantile epiphyses into adult females arrests their oestrous cycles in the stage of dioestrus.

2. Subcutaneous or intraperitoneal implantations of infantile epiphyses of the rat or calf into adult female rats do not influence their oestrous cycle.

3. Daily subcutaneous injections of epiphyseal extracts into adult female rats continued for 4 days do not affect the cycle.

4. No indication can be found that the infantile pineal body produces a hormone inhibiting the oestrous cycle of the rodents.

I wish to express my best thanks to Prof. D. E. Derry for his interest in the work, to Prof. G. V. Anrep for his constant encouragement, and to Dr A. A. Tarkhan for the suggestion of this research and for the invaluable help and criticism which he gave me during its performance.

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A NOTE ON THE MICROSCOPIC STRUCTURE OF ENAMEL

By J. D. KING
Beit Memorial Research Fellow

From the University Field Laboratories, Sheffield

In the course of experiments designed to test the effects of various food deficiencies on the development of the teeth and jaws of rats and dogs, thin microtome sections of enamel in different stages of formation were obtained from material which had not been submitted to prolonged decalcification nor mechanically agitated in the decalcifying fluid (5 % nitric acid) employed. In certain regions of the teeth, longitudinal and transverse paraffin sections, 7·5 μ in thickness and stained with aniline blue and orange G or haematoxylin and eosin, revealed a considerable bulk of retained enamel matrix. The following is a brief account of the appearance of the enamel under such conditions. Two of the sections illustrated have formed part of a demonstration at the Annual Meeting of the British Dental Association (1937).

1. RAT'S INCISOR

The incisor of a rat, being of persistent growth, is of course one of the most useful teeth for studying the different stages of enamel development. Fig. 1 is a diagrammatic drawing of a longitudinal, ground (undecalcified) upper incisor. If the tooth had been immersed for 2–3 weeks in alcoholic carmine before grinding, the portion of enamel posterior to the oblique line at *J* would have taken up the stain, while the enamel anterior to this would have remained unstained. In other words, the latter is more fully calcified than the former. It is of course obvious that such a distinction between "forming" and "formed" enamel is exaggerated, since the one merges gradually into the other. However, it serves the purpose of indicating approximately the part of the enamel from which Figs. 2–6 were made; that is, from the tissue in the immediate vicinity of *J*.

Fig. 2 is a photomicrograph of a rat's incisor in transverse section, decalcified and stained with haematoxylin and eosin. The enamel matrix has been retained and its so-called fibrous structure is evident. In Figs. 3 and 4 a higher magnification of the marked rectangular area in Fig. 2 is shown, in which it can be seen that the tissue is divided into inner and outer portions

according to the direction and arrangement of the prisms.[1] Thus, approximately the inner two-thirds consists of obliquely directed interlacing bundles of prisms, the number of prisms per bundle appearing to vary in different parts of the tissue; it should be noted that the individual prisms themselves do not interlace. On the other hand, at the junction of the inner two-thirds

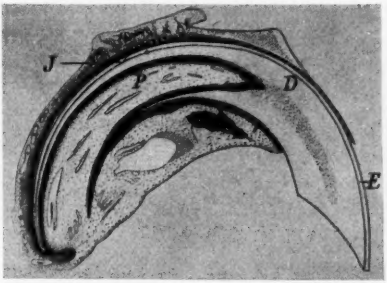

Fig. 1. Diagram (× 6) of ground longitudinal section of rat's upper incisor untreated by acid. If stained with alcoholic carmine, the enamel posterior to *J* would be coloured and that anterior to *J* uncoloured. *E*, enamel; *D*, dentine; *P*, pulp.

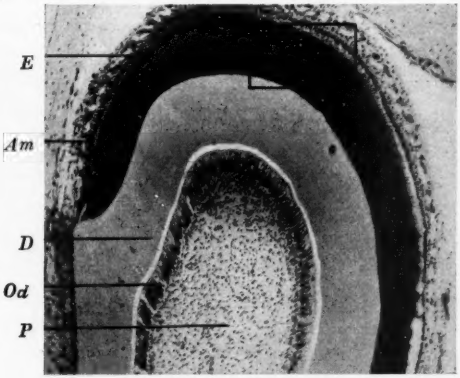

Fig. 2. Photomicrograph (× 100) of transverse section of rat's incisor at level just posterior to *J* in Fig. 1. Decalcified section stained with haematoxylin and eosin. Note retention of enamel matrix. *E*, enamel; *Am*, ameloblasts; *D*, dentine; *Od*, odontoblasts; *P*, pulp.

and outer third both the bundles and their component prisms turn sharply to run parallel to one another without interlacing and in a direction which, in the central axis of the transverse section, is at right angles to the tooth surface.

[1] The term "prisms" for the principal components of most enamels has recently been criticized by some investigators. It has, however, been retained here since evidence is still lacking as to the exact morphology, composition and function of these structures.

As seen in Fig. 4, the farther away from the central axis, the more oblique are the prisms of the outer layer.

Figs. 5 and 6 show the appearance of such enamel when cut longitudinally in a plane roughly corresponding to a line from *X* to *Y* (Fig. 3) and still in the vicinity of *J* (Fig. 1); the specimen was stained with aniline blue and orange G. Fig. 6 is a continuation of Fig. 5 extending more anteriorly towards the tip of the tooth. Thus, the left hand side of Fig. 5 represents the enamel in its earlier and less calcified stage of development and the right hand side of Fig. 6 the tissue at a later and more calcified stage. It will then be noted that the arrangement of the prisms becomes progressively less regular, both in the inner and outer parts of the enamel, as the more fully formed tissue is approached; this is presumably due to the physical or chemical action (or both) of the decalcifying fluid employed. It is then obvious that the more typical arrangement of the enamel prisms is to be seen in Fig. 5 in the region below *AB*. Here columns of prisms are running slightly obliquely from the dentine towards the enamel periphery for some two-thirds of the tissue, and between these are other structures at a still more oblique angle. Although in Fig. 5 some of the prisms appear to pursue a spiral course, as at *IL*, use of the microscope fine adjustment clearly shows that no such spiral arrangement actually exists but that the columns (Fig. 5, *PT*) are in reality made up of a number of prisms in transverse section. The other structures (*PL*) between the columns are prisms cut more or less longitudinally. On reaching the outer third of the enamel, the abrupt change in direction of both sets of prisms corresponds to that observed in Figs. 3 and 4. Thus in Fig. 5 the outer enamel layer is composed of obliquely cut prisms derived from both the transverse and longitudinal prisms of the inner layer.

2. DOG'S PREMOLAR (UNERUPTED)

Decalcified sections were also made from a few unerupted premolar teeth of dogs. Figs. 7 and 8 are photomicrographs of the inner part of the enamel of one of these specimens, cut vertically in a bucco-lingual plane and stained with haematoxylin and eosin. Fig. 7 is from the inner part of the occlusal enamel and Fig. 8 that of the buccal enamel. The very complex nature of the prism arrangement is indicated by the different directions in which the prisms are cut in a section as thin as $7{\cdot}5\,\mu$. The author has not yet determined the enamel pattern of the dog's teeth, nor have sufficiently good preparations been made for studying the histological differences—if any—between the enamel periphery and the rest of the tissue. Technical difficulties have to a large extent obscured the issue, especially in the outermost enamel where an unfortunate tendency to contract or tear during fixation, decalcifying, cutting and staining is so often encountered.

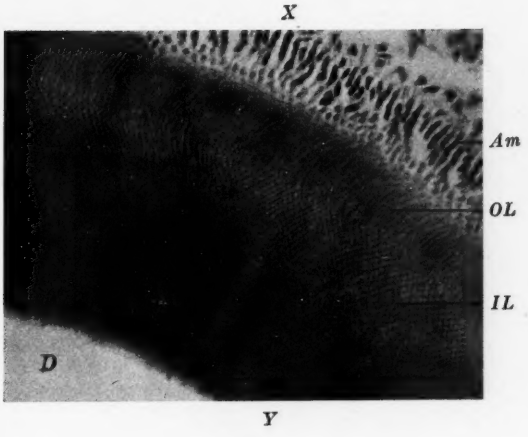

Fig. 3.

Fig. 4.

Figs. 3, 4. Photomicrographs ($\times 500$) of marked rectangular area in Fig. 2. Fig. 4 is a buccal continuation of Fig. 3. Note differentiation of enamel into two layers. In the inner layer bundles of prisms interlace obliquely and in the outer layer run parallel to one another. *Am*, ameloblasts; *OL*, outer enamel layer; *IL*, inner enamel layer; *D*, dentine.

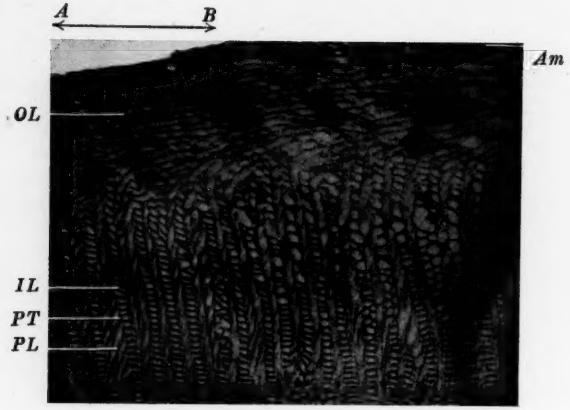

Fig. 5.

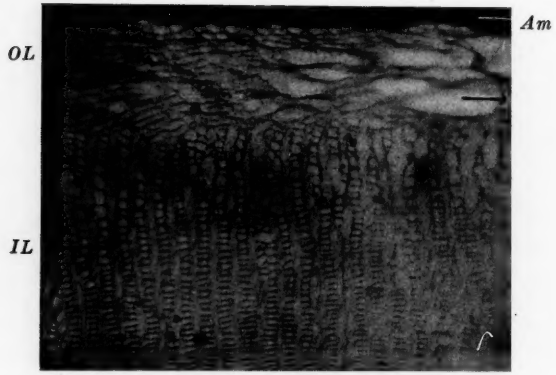

Fig. 6.

Figs. 5, 6. Photomicrographs ($\times 500$) of longitudinal section of rat's enamel in region *J* (Fig. 1) and plane *XY* (Fig. 3). Decalcified section stained with aniline blue and orange G. Fig. 6 is a continuation of Fig. 5 extending more anteriorly towards the tip of the tooth, indicated by direction of arrow (Fig. 6). The tissue beneath *AB* (Fig. 5) is in the earlier stage of development and shows typical prism arrangement. *OL*, outer enamel layer; *IL*, inner enamel layer; *Am*, ameloblasts; *PT*, prisms cut transversely; *PL*, prisms cut more or less longitudinally.

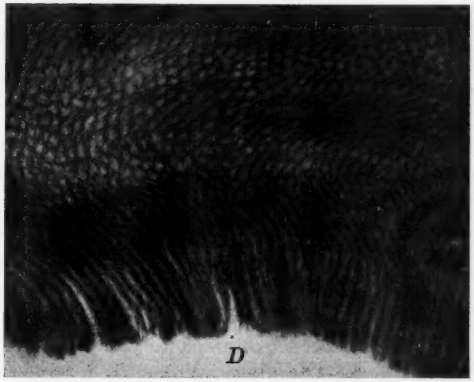

Fig. 7.

Fig. 8.

Figs. 7, 8. Photomicrographs (× 500) of vertical bucco-lingual section of inner part of enamel of unerupted second premolar of a dog. Decalcified section stained with haematoxylin and eosin. Note different arrangement of prisms beneath occlusal surface (Fig. 7) and buccal surface (Fig. 8). *D*, dentine.

DISCUSSION

With regard to the enamel structure of the rat's incisor, the investigations of J. Tomes (1850), Leon Williams (1896), C. S. Tomes (1914) and J. H. Mummery (1924), all of whom employed ground (undecalcified) sections, have been to a large extent confirmed. It is believed, however, that the use of decalcified material has enabled the present author to obtain a clearer conception of the arrangement of the enamel prisms both in transverse and longitudinal section. The occurrence of the prism "serrations" described by J. Tomes and C. S. Tomes has not been confirmed and support is given to Mummery's view that any apparent serrations were probably mistaken for interprismatic substance. It is of interest that, although the incisor and molar teeth of the rat are used for such different purposes, the arrangement of the enamel prisms appears to be identical, although in the molar teeth at certain stages of development the typical pattern may be somewhat obscured.

Insufficient material has been examined to justify any conclusions as to the enamel pattern of the dog's premolar. While it is notable that Figs. 7 and 8 show a similar arrangement of the prisms to that illustrated by Allen (1930), again further work is necessary before his suggested system of alternating circular and longitudinal enamel prisms can be discussed. It is obvious that the enamel of the rat and the dog differ very considerably in microscopic structure, and J. Tomes, C. S. Tomes, Carter (1922) and others have stressed the classification of enamels according to the type of animal to which they belong. For example, Carter states that: "In the long and well-authenticated series of creatures comprising the ancestry of the Horses, the micro-structure of the enamel does not change, in spite of the modification of the teeth from the brachyodont form in *Eohippus* through all the progressive stages leading up to the complex hypsodont molar teeth of recent horses." It seems probable that the different enamel patterns which today characterize the teeth of the various mammalian families, such as the Rodents, Ungulates, Carnivora, etc., were evolved at an earlier geological period than were the species which now comprise these families. This is suggested by the fact that the enamel pattern was much the same in the ancestors of the family as it is in the present day descendants, although, as in the horse, the teeth themselves may have evolved from a brachyodont to a hypsodont type.

It is hoped that the special decalcifying technique devised by Malleson (1924, 1925) will be applicable to the further study of enamel in various stages of development, both in animals and in man, and that by this means some useful knowledge may be obtained of the histological structure of the tissue in which dental caries is first detected.

SUMMARY

1. A brief account is given of the microscopic structure of the enamel of the rat's incisor and the dog's premolar.

2. The arrangement of the enamel prisms in transverse and longitudinal sections and the differentiation of the enamel into two layers in the *rat's incisor* is described.

3. The appearance of developing enamel in the *dog's premolar* is illustrated. Investigation of its enamel pattern is now in progress.

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THE EFFECT OF OESTRADIOL MONOBENZOATE ON THE GONADS, ENDOCRINE GLANDS AND MAMMAE OF LACTATING RATS

By P. BACSICH

Department of Anatomy, University of Glasgow

AND S. J. FOLLEY

National Institute for Research in Dairying, University of Reading

INTRODUCTION

Studies on the endocrinology of lactation now in progress at the National Institute of Research in Dairying gave us an opportunity of observing the effects of high daily doses of oestradiol monobenzoate not only on the mammary glands (in which our main interest lay) but also on the endocrine glands and ovaries of lactating rats.

The effect on the hypophysis of administration of oestrogens has been fairly extensively studied both from the standpoint of functional changes and from that of histological changes. The pituitary implantation technique has shown that one consequence of oestrogen treatment is a decrease in the gonadotropic potency of the pituitary (Meyer *et al.* 1929–30), while evidence pointing in the same direction has been obtained by Jones & MacGregor (1936) who observed a decrease in the urinary excretion of gonadotropic hormones after oestrogen treatment in the human. It is interesting to note that the work of Fevold *et al.* (1936) indicates that the luteinizing potency of the pituitary is, on the other hand, increased by administration of oestrogens.

The changes in the gonads and endocrine glands which follow the administration of oestrogens are reminiscent of those following hypophysectomy. Thus in the young male, administration of oestrogens is followed by failure of the testes to develop (Moore & Price, 1932; Zondek, 1936*a*), while in the mature male the testes undergo atrophy (Halpern & D'Amour, 1936). In the female the dosage of oestrogen appears to determine to some extent the effect produced on the ovary since after treatment with low levels of oestrogen Meyer *et al.* (1929–30), Doisy *et al.* (1930–1), Selye *et al.* (1935) and Zondek (1936*a*) observed ovarian atrophy, while with higher doses, Selye *et al.* (1935) observed enlargement of the ovaries due to increase in the size of the corpora lutea. Injection of oestrogens also diminishes the growth rate in young animals (Zondek, 1936*b*) and, when relatively high doses are given, completely or almost completely inhibits milk secretion in lactating animals (see Folley & Kon, 1937, for numerous references). It may further be added that high doses of oestrogens markedly inhibit the response of the pigeon crop gland to prolactin

(Folley & White, 1937), a response which has been shown by Schooley *et al.* (1936) to be much less, for a given dose of prolactin, in hypophysectomized pigeons than in intact ones.

Changes in the adrenals following oestrogen administration have been studied by Selye *et al.* (1935) who reported that such treatment was followed by adrenal enlargement in female rats, though the effects were hardly perceptible in the male. Leiby (1933) reported adrenal enlargement in ovariectomized female rats after oestrogen treatment, while Cramer & Horning (1936*a*) described degenerative changes in the adrenal cortex of the mouse following oestrogen administration, again reminiscent of post-hypophysectomy effects. Later, Cramer & Horning (1937) described a process of "brown degeneration" occurring in the adrenal cortex of the mouse following the administration of certain oestrogens.

There is reason to believe that the effects on the thyroids of prolonged administration of oestrogens resemble in many respects those associated with the hypothyroidism which follows hypophysectomy. According to Bialet-Laprida (1933) and Benazzi (1933) the thyroid epithelium after oestrogen administration is low and the vesicles large and their lumina are filled with colloid. Bialet-Laprida (1933) reported that oestrogen administration led to a decrease in weight of the thyroids of the rat. It may be noted that Montpellier & Chiapponi (1930) could detect no changes in the thyroids (nor adrenals) after oestrogen treatment in the rat, while Leiby (1933) reported an increase in the weight of the thyroids in ovariectomized rats after oestrogen treatment.

Thy hypofunction of the pituitary due to oestrogen administration is usually accompanied by enlargement of the pituitary gland itself. Zondek (1936*c*) could only observe this phenomenon in the male rat, though in one female which had received injections of oestrogen he described a huge pituitary tumour. Selye *et al.* (1935) described enlargement of the pituitary in female rats after oestrogen administration and McEuen *et al.* (1936) in males. The latter workers also reported the occurrence of anterior lobe adenomata following oestrogen treatment. Cramer & Horning (1936*b*) and Halpern & D'Amour (1936) give detailed descriptions of the histological changes induced in the pituitary by oestrogen treatment.

The general interest and importance of this subject, together with the fact that all investigators have not agreed at all points, on the effects on the endocrine system which follow from prolonged administration of oestrogenic substances—possibly on account of differences in dosage and other experimental conditions—seemed to justify the recording of certain of our observations.

EXPERIMENTAL

The rats were hooded Norway rats belonging to the colony maintained in this Institute, full facilities for working with which were generously accorded by Dr S. K. Kon. Their ages varied between 8–13 months and at the time of the experiments most were experiencing their second or third lactations,

though a few were in their first or fourth lactations. Those selected for ovariectomy were operated upon on the day following parturition, the ovaries being removed through dorsal incisions under avertin anaesthesia (1 ml. of $2\frac{1}{2}$ % avertin per 100 g. body weight intraperitoneally). Table I shows the size of the groups used and the experimental procedure accorded to each. The oestrogen used was Progynon B Oleosum Forte (Schering), for generous supplies of which we are indebted to Dr H. Neumann of Messrs Schering, Ltd. The effect of the experimental procedure on the efficiency of lactation in these rats has been described previously (Folley & Kon, 1937).

Table I

Group	No. of rats in group	Experimental treatment
1	6	20 daily subcutaneous injections of 0·2 ml. sesame oil beginning on the 1st day of lactation.
2	4	Ovariectomized rats given 20 daily subcutaneous injections of 0·2 ml. sesame oil beginning on the 1st day of lactation
3 (*a*)	2	12 daily subcutaneous injections of 1 mg. oestradiol monobenzoate in 0·2 ml. sesame oil beginning on the 5th day of lactation
3 (*b*)	4	20 daily subcutaneous injections of 1 mg. oestradiol monobenzoate in 0·2 ml. sesame oil beginning on the 1st day of lactation
4	3	Ovariectomized rats given 20 daily subcutaneous injections of 1 mg. oestradiol monobenzoate in 0·2 ml. sesame oil beginning on the 1st day of lactation

All rats were killed on the day of weaning (22nd day of lactation) and the endocrine glands dissected out and weighed, paired glands being weighed together. They were then fixed in Bouin's fluid, embedded in paraffin, and sections cut at 5 μ and stained with haematoxylin and eosin. Special techniques for fixation and staining of adrenals and pituitaries were not used. At autopsy, specimens of the mammary glands were taken and prepared for histological study.

The weights of the endocrine glands

In Table II are given the mean weights of the endocrine glands for each group together with the highest and lowest values in each group.

Hypophysis. The mean weight of the pituitary in the intact rats which received injections of oestradiol monobenzoate (group 3*b*) is almost three times that of the controls, the heaviest pituitary in the former group weighing 48·3 mg. against a control mean of 13·8 mg. The statistical significance of the difference between the mean values was estimated in this and other cases by Fisher's (1934) "*t*" test, the probability *P* that a difference at least as great as the observed difference should have arisen purely as an accident of random sampling coming out at 0·001, i.e. 1 in 1000. Similarly in ovariectomized rats the hypophysis after oestrogen treatment was again on the average almost three

Table II. *Effect of oestrogen treatment on the weights of the endocrine glands and ovaries of lactating rats. All weights in mg.*

Group no.	Treatment	Hypophysis			Adrenals		
		Highest	Lowest	Mean	Highest	Lowest	Mean
1	Sesame oil (control)	17·3	9·7	13·8	102·3	69·9	88·3
2	Ovariectomized sesame oil	17·2	13·6	15·1	93·8	69·8	83·6
3 (*a*+*b*)	Oestradiol monobenzoate	48·3	27·9	36·9	100·0	62·6	82·5
3*b*	Oestradiol monobenzoate	48·3	27·9	38·0	100·0	71·9	87·3
4	Ovariectomized oestradiol monobenzoate	52·9	30·4	42·9	100·5	85·7	91·6

Group no.	Treatment	Thyroids			Ovaries		
		Highest	Lowest	Mean	Highest	Lowest	Mean
1	Sesame oil (control)	55·6	35·5	47·8	92·9	62·3	76·9
2	Ovariectomized sesame oil	59·2	33·8	44·6	—	—	—
3 (*a*+*b*)	Oestradiol monobenzoate	80·7	31·7	46·4	160·1	93·0	120·7
3*b*	Oestradiol monobenzoate	44·3	31·7	38·0	126·3	93·0	107·8
4	Ovariectomized oestradiol monobenzoate	45·6	28·4	37·5	—	—	—

times heavier than in the case of the ovariectomized controls, the heaviest pituitary observed in the whole series, namely one weighing 52·9 mg., occurring in the former of these two groups. Once more in spite of the small size of the groups the difference between these two mean values was highly significant ($P=0{\cdot}004$). The mean weight of the hypophysis in ovariectomized rats was slightly greater than in the normal controls and also in the ovariectomized rats receiving injections of oestradiol monobenzoate as against the mean for normal rats receiving similar injections, but in neither case was the difference statistically significant.

Our results, therefore, agree with those of Selye *et al.* (1935) and others (for bibliography see Lauson *et al.* 1937) in showing in contradistinction to the results of Zondek (1936*b*) that oestrogen administration leads to pituitary enlargement in female rats. It should be noted, however, that the dosage in our experiments was much greater than that used by Zondek.

Ovaries. The mean weight of the paired ovaries in the rats which had been injected with oestradiol monobenzoate (group 3*b*) was about 50 % greater than the mean control weight, in agreement with the results of Selye *et al.* (1935). This effect was highly significant since $P=0{\cdot}007$.

Adrenals. No statistically significant differences could be found between the mean weights of the adrenals for the various groups of rats. The adrenals from both groups of animals which received oestrogen injections differed strikingly in appearance from those of both groups receiving sesame oil alone in that in the former groups the adrenals were dark red, resembling kidney tissue in colour. This suggested that oestrogen administration produces hyperaemia of the cortex, a supposition which was confirmed by histological examination (see below).

Thyroids. In both normal and ovariectomized rats the mean weight of the thyroids of oestrogen treated animals was less than that of the controls. In the

ovariectomized groups the difference between the mean values had no statistical significance, but in the case of normal animals the probability that the observed depression in the mean weight of the thyroids of the rats receiving injections of oestrogen was due to a chance of random sampling was only 7 in 100. This, though somewhat larger than the usually accepted criterion of statistical significance, namely $P \leqslant 1$ in 20, nevertheless strongly suggests, in agreement with Bialet-Laprida (1933), that the pituitary suppression due to oestrogen administration is accompanied by a decrease in thyroid size, at any rate when high doses of oestrogen are given.

Histological changes in the endocrine glands

Hypophysis. In both groups of rats which had received injections of oestradiol monobenzoate, histological examination showed that in the pars glandularis the chromophobe cells were increased in size and number at the expense of eosinophiles and basophiles. In the ovariectomized rats signet-ring cells only occasionally could be distinguished. There was marked hyperaemia of the anterior lobe together with numerous haemorrhages and some vacuolar degeneration. These findings are in general similar to those reported by Cramer & Horning (1936*b*) in mice and by Halpern & D'Amour (1936) in rats.

Adrenals. In the oestrogen treated groups, histological examination showed evidence of hypertrophy of the adrenal cortex accompanied by hyperaemia. Haemorrhagic cysts could occasionally be distinguished in the cortex. In some specimens the adrenal capsule had burst. Frequently, vacuolization of the cortical cells was observed. This was probably due to the removal of the lipoids in the process of embedding.

In all specimens whether from oestrogen treated animals or from controls, the zona glomerulosa, zona fasciculata and zona reticularis could be clearly distinguished. Inside the zona reticularis an almost continuous layer of large rounded cells with well marked nuclei and eosinophilic cytoplasm could be seen (Pl. I, figs. 1–3). These cells were clearly distinguishable from those of the rest of the reticularis on account of their size. Occasionally isolated groups of them were to be found in the medulla. This layer was somewhat diminished in the animals which had been given injections of oestrogen.

The special cytology, life history and experimental behaviour of these cells require further study, but even at this stage we consider that these cells belong to the cortex. In our preparations the cells in question are characterized by clearly defined boundaries, they stain strongly with eosin, their nuclei show a characteristic chromatin granulation and usually there is a well recognizable nucleolus situated in the middle of the nucleus. The cell boundaries of the medulla cells, on the other hand, are *ill* defined and the chromatin granules are precipitated on the surface of the nuclear membrane and the nuclei are devoid of nucleoli. At present it is difficult to say whether or not these cells which we propose, for the time being, to call "*Y*" cells, are equivalent to those mentioned by Howard (1938) and Anderson & Kennedy (1932). On the other hand, it is

very likely that the same cells ("δ" cells) were observed by Bourne (1934) in the adrenal of the opossum.

Thyroids. The thyroids of the oestrogen treated rats showed histological changes as compared with the controls which were in general indicative of hypofunction. The acini contained more colloid than in the controls and the epithelium was low (Pl. I, figs. 4, 5).

Ovaries. After oestrogen treatment the ovaries contained many atretic follicles. Frequently large cystic follicles were seen, in one instance encased in a layer of luteal cells. Proliferation of the membrana granulosa cells was commonly observed. The corpora lutea were in many cases much larger than normal and often miliary corpora lutea were seen (Pl. II, fig. 1). There were even isolated luteal cells scattered in the ovarial stroma (Pl. II, fig. 2). It therefore appears that, under some circumstances, luteal cells can originate from ovarian cells other than cells of the membrana granulosa.

The histology of the mammary glands

Histological sections of the mammary glands of all groups rather surprisingly presented much the same appearance. Even in the groups which had received oestrogen treatment there were few signs of the involution which would be expected in view of the lactation inhibition caused by high doses of oestrogen (see Folley & Kon, 1937). All the mammary glands were examined for infiltration of the alveolar epithelium by leucocytes and lymphocytes and also for pycnosis and karyolysis, but none of these signs of involution could be discovered with certainty in any specimen whether from oestrogen treated or control rats. There were, in some preparations, indications of a slight increase in the proportion of stroma to parenchyma, but there was relatively little disintegration of the alveoli. Some acini were distended with coagulated secretion, the epithelium being low, while others in the same specimen were empty with high epithelium. In most fields alveoli representative of various stages of the secretory cycle could be distinguished (Pl. II, figs. 3, 4).

DISCUSSION

In many respects our results are in accord with those obtained by previous workers discussed above, a noteworthy point being the striking enlargement of the hypophysis, namely 2–3 times normal, which is produced by administration of high doses of oestrogen, in this case, to lactating rats. In our experiments the pituitary enlargement appeared to be accompanied by suppression of the secretion, by the anterior pituitary, of the thyrotropic hormone as evidenced by histological alterations in the thyroid gland and decrease of its size (see also Bialet-Laprida, 1933 and Benazzi, 1933). By our technique no significant changes in the weight of the adrenals following oestrogen treatment could be detected, though this does not preclude the possibility that such changes would have been observed had larger groups of rats been used. We confirm Cramer & Horning (1936*a*) as regards the occurrence of degenerative

changes in the adrenal following oestrogen treatment. In addition, the treatment caused hyperaemia of the cortex to such an extent that haemorrhages were numerous. Indeed, the adrenals of our oestrogen treated rats were dark red in colour as distinct from the brownish yellow adrenals of the controls.

Despite the suppression of the anterior lobe gonadotropic hormone secretion which undoubtedly follows treatment with oestrogens, the ovaries of our animals were considerably heavier than those of normal controls, due not only to increase in the size of the corpora lutea existing before the beginning of treatment (see also Selye *et al.* 1935) but also probably to the formation of new corpora in the ovarian tissue.

As described in a previous paper (Folley & Kon, 1937), in our animals the administration of oestrogen suppressed lactation, but as the present histological examination shows, marked involution of the mammary gland did not follow. The loss in body weight which was previously shown to accompany the administration of oestradiol monobenzoate to these rats may therefore be partly due to a generalized effect on the body, probably similar in origin to the emaciation which follows hypophysectomy in the adult, instead of being, as was suggested earlier, wholly due to involution of mammary tissue. Since the mammary glands were not removed and weighed at autopsy it is, however, impossible to say whether or not the mammae of the oestrogen treated rats had lost weight during the period of oestrogen administration. It is surprising to find that even in cases where, due to the inhibitory effect on lactation of oestrogen administration, all of the young had died before weaning and where, therefore, no suckling had occurred for some days prior to autopsy, the mammae showed practically no signs of involution. It is well known that mammary involution rapidly sets in if milk is not withdrawn and therefore accumulates in the mammary gland. The conditions in the mammae of our oestrogen treated rats were, however, somewhat different from those existing after weaning in the normal animal, since in view of recent developments (see Nelson, 1936) it is reasonable to suppose that the oestrogen at one and the same time suppressed the secretion of lactogenic hormone by the anterior pituitary (thereby largely removing the stimulus to milk formation) and preserved the existing mammary parenchyma against degeneration.

SUMMARY

1. The effect of high daily doses (1 mg.) of oestradiol monobenzoate on the weights and histology of the endocrine glands and ovaries and on the histology of the mammary glands of lactating rats has been studied.

2. The treatment caused marked enlargement of the pituitary in both intact and ovariectomized animals accompanied by hyperaemia and haemorrhages. The chromophobe cells of the pars glandularis were increased in size and number as a result of oestrogen administration.

3. The thyroids of oestrogen treated rats were lighter than those of controls (P = approximately 1 in 14). In both ovariectomized and intact rats treated

with oestrogen the histological picture of the thyroid was typical of hypoactivity.

4. No significant alteration in the weights of the adrenals as a result of oestrogen administration could be detected in either intact or ovariectomized rats by our technique. The treatment caused hyperaemia in the adrenal cortex and also degeneration. In the adrenals of all the rats examined by us a layer of large eosinophilic cells with well defined cell boundaries ("*Y*" cells) could be distinguished inside the zona reticularis. Islets of such cells were scattered in the medullary tissue. This layer was somewhat diminished in oestrogen treated animals.

5. The ovaries of treated rats were heavier than those of controls due to an increase in the size and number of the corpora lutea. Such ovaries contained miliary corpora lutea and isolated luteal cells in the ovarial stroma.

6. Though the treatment had caused inhibition of lactation, the mammary glands of oestrogen treated rats showed few signs of involution even in cases where no suckling had occurred for some days prior to autopsy.

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 1. Section of adrenal of control lactating rat showing, enclosed by inked lines, the zone of "*Y*" cells between the zona reticularis and the medulla. 19 ×.

Fig. 2. High power view of region enclosed within the larger rectangle shown in Fig. 1. The large eosinophilic "*Y*" cells form a zone between the zona reticularis at the top of the figure and the medulla at the bottom. 360 ×.

Fig. 3. High power view of region enclosed within the smaller rectangle in Fig. 1 showing isolated groups of "*Y*" cells in the medulla. 510 ×.

Fig. 4. Section of thyroid gland of control lactating rat. 80 ×.

Fig. 5. Section of thyroid gland of lactating rat after treatment with oestradiol monobenzoate. Note the accumulation of colloid in the vesicles. 80 ×.

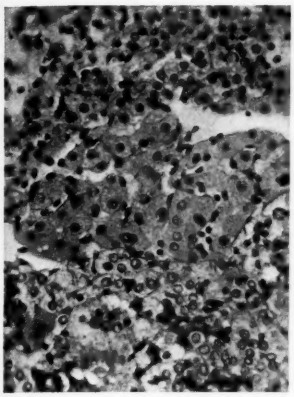
Plate II

Fig. 1. Section of ovary of lactating rat after treatment with oestradiol monobenzoate showing miliary corpora lutea and atretic follicles. 142 ×.

Fig. 2. High power view of section of ovary of lactating rat after treatment with oestradiol monobenzoate showing luteal cells in the ovarial stroma. 380 ×.

Fig. 3. Section of the mammary gland of control lactating rat killed at weaning. 80 ×.

Fig. 4. Section of the mammary gland of rat which had received daily injections of oestradiol monobenzoate during lactation. All the young of this animal died of inanition three days before she was autopsied, 22 days after parturition. There are relatively few signs of involution. The alveoli are mostly filled with coagulated secretion. 80 ×.



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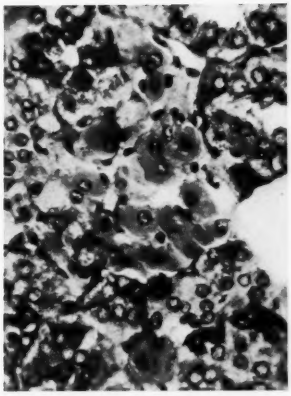

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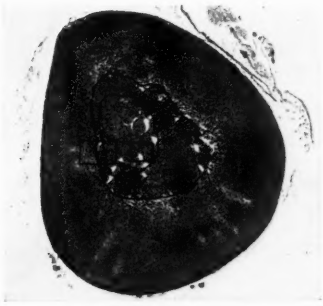

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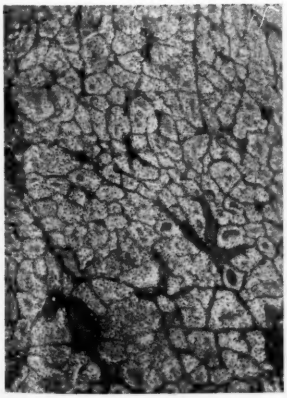

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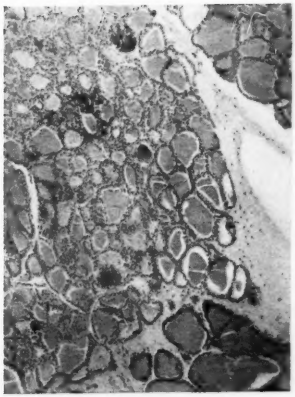

Fig. 5.

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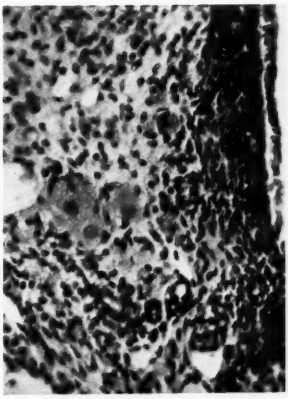
Fig. 1.



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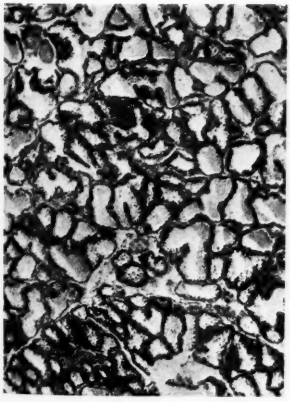

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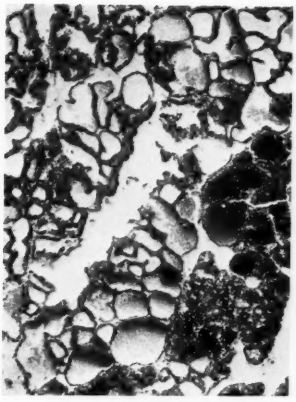

Fig. 4.

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ANENCEPHALY AND RHACHISCHISIS POSTERIOR, WITH THE DESCRIPTION OF A HUMAN HEMICEPHALUS OF 18 MM.

By W. F. H. STRÖER and A. van der ZWAN

From the Anatomical Laboratory and the Department of Neurology and Psychiatry of the University of Groningen, Netherlands

INTRODUCTION

The malformations of the central nervous system, known as anencephaly, hemicephaly, rhachischisis posterior, spina bifida, etc., may take their origin in two different ways.

(1) They may originate from a non-closure of the neural plate. The persistence of the neural plate condition may involve the whole length of the nervous system, or it may be localized in some definite region, e.g. in the anterior head region (Fig. 1A), or in more posterior parts of the brain or spinal

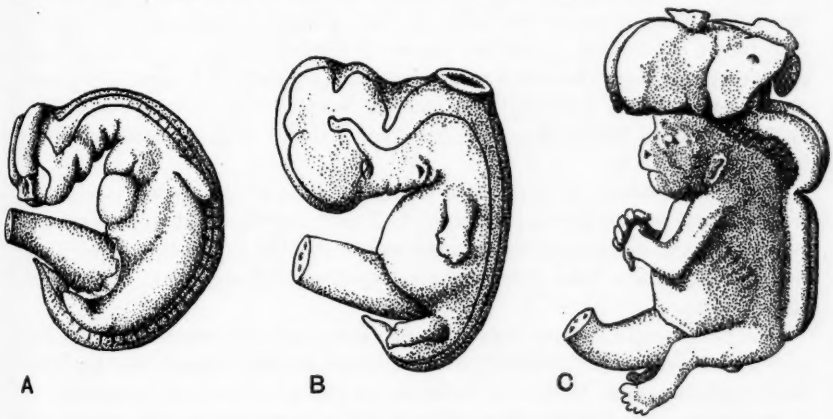

Fig. 1. Three embryos, showing: A, a normally closed central nervous system, with the exception of the head region (pig of 8 mm., after Baxter & Boyd); B, the same for the upper region of the spinal cord (human case of Sternberg); C, central nervous system open over entire length (human embryo of 22 mm.).

cord (Fig. 1B). Fig. 1C shows an example of a rare case of non-closure of the neural tube over its whole length.

In later stages of development, the part of the central nervous system in which fusion fails, shows a tendency to disappear. Lebedeff (1881) and

Sternberg (1929) have illustrated this gradual disappearance. The degeneration of the central nervous system occurs only after the medullary plate has given rise to the peripheral nerves. As a result a full-term foetus may develop in which these parts of the brain or spinal cord are absent, while the peripheral nerves are well developed. Belonging to this category are several types of abnormality described as anencephalus, hemicephalus, spina bifida, rhachischisis posterior, etc.

The question arises—what factor is responsible for the closure of the neural tube? By experimental research it has been shown that the underlying mesoderm (the "Urdarmdach") plays an important role in the formation of the neural plate and its further development. On the other hand, a defective condition of the underlying mesoderm leads to a defect in the development of the central nervous system: Lehmann (1926, 1938) has demonstrated that, if parts of the presumptive mesoderm are extirpated before invagination, those parts of the central nervous system lying above these artificial defects of the organizer show corresponding deficiencies. As a consequence, besides the malformations of the central nervous system, abnormalities may be expected in the underlying skeleton and musculature which take their origin from the mesoderm of the organism. These latter abnormalities should be considered as the primary cause of the deformities of the central nervous system.

As to the aetiology of the disturbance in the "Urdarmdach", with its manifold consequences, we can only say that, at least in some cases, a hereditary factor is involved. Cases are known of familial occurrence of synostosis of cervical vertebrae combined with spina bifida (malady of Klippel-Feil), and also of spina bifida with vertebral defects in other regions (Schwarzweller, 1937). De Vries (1915) also mentions some families with anencephaly as a hereditary factor.

(2) Malformations of the central nervous system of the second type arise from an early local or general hydrops. In these cases the closure of the neural tube is normal, but after closure an excessive collection of cerebro-spinal fluid in the tube causes local enlargements (types of hydrocephalus, myelocele, spina bifida).

Kr. Bonnevie has shown that an excess of the fluid may escape through the ependymal roof of the fourth ventricle, either by passing through the thin wall or through foramina. In this way blebs are formed which shift along the surface of the body of the embryo to different places where their effect becomes manifest in malformations. If the excess of fluid is not able to leave the ventricles of the brain, it gives rise to a swelling of the neural tube, and a thinning of the wall. The thinning of the wall may lead to its degeneration, giving rise to the appearance of a partly open tube, such as has been described in the first category. The aetiology of the excess of fluid in the neural tube is not known, but it appears that hereditary factors play an important role, as has been shown by the experimental work of Kr. Bonnevie (1934, 1935).

MATERIAL

The embryo to be described was presented to the Anatomical Institute of the University of Groningen by Prof. Deelman several years ago. As far as is known, it was one of a series of abortions of one woman. When received, it was preserved in 10% formalin. Ordinary photographs, stereophotographs and drawings were made. Subsequently it was embedded in celloidin and cut into transverse sections (25 μ thickness) which were stained with haematoxylin-eosin. From a histological point of view the material was not ideal, but the different tissues and organs could be well distinguished. Both graphic reconstructions and wax models were made to clarify the more difficult relations. We here express our thanks to one of our students, Mr Rinsma, who made a wax model of the greater part of the axial skeleton.

SURFACE ANATOMY

The crown-rump length of the embryo is 18 mm. Externally several abnormalities are observable. The most striking of these malformations is

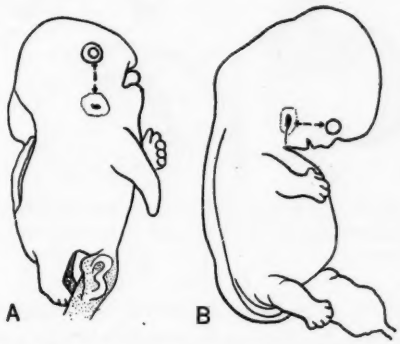

Fig. 2. Lateral view of the anencephalic embryo (A) and of a normal embryo of the same length (B, after Keibel & Elze).

found in the mid-dorsal region, stretching from the neck to the pelvic region (Fig. 5). Here the skin and subcutaneous tissue are absent, and there are exposed to view a median blood vessel and on either side a row of spinal ganglion. The spinal cord is absent. Rostrally, two larger masses of brain tissue are present (Fig. 5).

Apart from the rhachischisis, the whole appearance of the embryo is abnormal, showing the typical features of anencephalus (Fig. 2). The extremities are well developed with the normal number of digits. A hare-lip is present on the right side.

MICROSCOPICAL ANATOMY

(1) *Central nervous system*

The microscopic sections show that in the abnormal dorsal region the spinal cord is absent, and confirm the identification of the spinal ganglia. The masses present in the cranial region (Fig. 5) are brain tissue which continues rostrally in the cranial cavity. These are the only remnants of the central nervous system.

Three parts of the brain can be distinguished, two lying antero-laterally (Fig. 6) and the other in a postero-median position. The first two represent the cerebral hemispheres, of which apparently a great part of the lateral wall and the roof are lacking (Fig. 6). This defect is due to an early non-closure of the neural tube as represented in Fig. 3. Instead of a closure, an

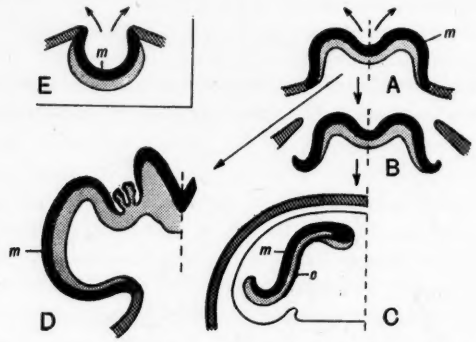

Fig. 3. Diagrams explaining the method of eversion of the medullary plate. From the normal E, through eversion, A is formed; from A, D may follow (Hunter's case), or B and C (our case). A, D and E after Hunter.

"extroversion" (Hunter, 1934–5) has occurred; as a result the inner layers are turned outside (from E to A). This process may lead to a condition represented in Fig. 4D (case of Hunter, 1934–5, and of Baxter & Boyd, 1938); in our own case it leads to a condition represented in Fig. 4C. Though the presumptive material for the lateral wall and roof is present, it does not reach its normal position. The free border of the lateral wall, showing signs of degeneration, is the place of former contact with the ectoderm. The interpretation here given is confirmed by the histological structure of the brain wall with its matrix, its intermediate layer and cortical layer (Fig. 6). It must be mentioned that the cortex is lacking in the ventral and lateral part of the hemisphere (Figs. 6 and 3C). No plexus chorioideus and no foramen Monroi can be observed; the falx cerebri is found in a normal position between the rudiments of the hemispheres.

The central portion of brain tissue contains many loose fragments (Fig. 6),

probably portions of the wall of the diencephalon and mid-brain; they are difficult to identify.

The hypophysis consists of a thick-walled pocket of tissue ending in two short posterior offshoots, as is normal for the adenohypophysis. The neurohypophysis is absent; no connexion of the hypophysis with brain tissue could be found. The glandular hypophysis is connected with the roof of the mouth

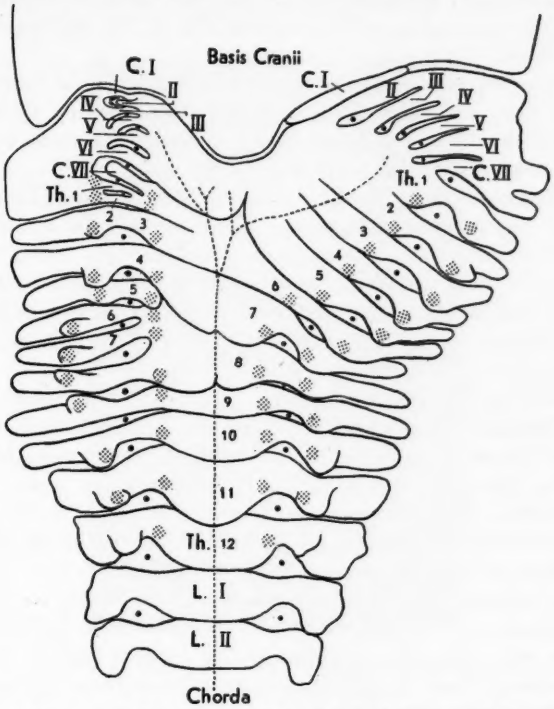

Fig. 4. Drawing of the wax model of the vertebral column. *L*, lumbar; *Th*, thoracic; *C*, cervica vertebra. Foveae costarii: stippled; spinal nerves: large dots.

cavity by means of a long but narrow stalk, running through the cartilaginous base of the skull.

The two internal carotid arteries run laterally of the pituitary body where they branch off into blood vessels and plexuses.

(2) *Peripheral nervous system*

(*a*) *Cranial nerves.*

(i) *Olfactorius.* This nerve is well developed on both sides. Coming from the mucous membrane of the olfactory sacs, nerve fibres can be traced over a certain distance. There is no olfactory bulb or tractus.

(ii) *Opticus.* On both sides optic nerves emerge from the eyeballs and run in postero-medial direction (Fig. 6, *n. II*). They both end near the optic foramen outside the chondrocranium.

(iii) *Eye muscle nerves.* Of the eye muscles the four recti and the obliquus inferior are present bilaterally. Of the associated nerves the n. oculomotorius and the n. abducens were observed. They pass caudalward, medial to the ganglion Gasseri and terminate behind the otic capsules in darkly stained masses of tissue containing no gray matter. The m. obliquus superior and its nerve are absent.

(iv) *Nervus trigeminus.* This nerve is well developed on both sides. The three chief rami with their different branches could easily be traced. The Gasserian ganglion is well developed. A motor root is present and its fibres lead to the primordia of the masticating muscles.

The connexion of the r. ophthalmicus with the Gasserian ganglion and the presence of the sympathetic ganglion ciliare cannot be established with certainty. The connexions of the maxillary and the mandibular nerves with the ganglia are quite apparent. Small nerve bundles pass to the sphenopalatine and otic ganglion. A strand of nervous tissue originates dorsal of the Gasserian ganglion, connecting the ganglia of the Vth, VIIth and VIIIth nerves.

(v) *Nervus facialis and nervus acusticus.* Both are present with their associated ganglia. The n. petrosus superficialis can be identified. The main trunk of the facial nerve cannot be followed within the mass of nerve and muscle fibres through which it passes. The eighth nerve is seen within the labyrinth capsule. The previously mentioned strand of nervous tissue which connects the different ganglia (of nerves V, VII and VIII) ends on same level as the foramen endolymphaticum.

(vi) The *ganglia* of the *ninth, tenth* and *eleventh nerves* form a complex gradually passing over into series with the cervical ganglia. One strong band of nerve fibres rises from this complex, finding its exit through the foramen lacerum posterius where it splits up into its different branches; in this region the sympathetic cervical ganglion is seen.

(vii) *Nervus hypoglossus.* It is present on both sides and finds its exit through the hypoglossal foramen (Fig. 6). It can be followed readily into the muscles of the tongue.

(*b*) *Spinal ganglia.*

All the spinal ganglia are evident.

The innervation of the musculature of the arm and leg is normal; the brachial and lumbar plexuses show their usual structure and relationships.

(*c*) *Sympathetic trunks.*

These are well developed bilaterally and can be followed along the vertebral column, showing the typical praevertebral enlargement in the region of the kidneys (plexus renalis and plexus coeliacus).

(3) *Sense organs*

The *nose* is well developed on both sides, but as previously mentioned, a hare-lip is present on the right side. The primordia of maxillo- and ethmoturbinale I and II are present on both sides (Fig. 6, *E.t.* and *M.t.*).

The eye. Both eyes are present; the lenses are well developed but show signs of degeneration (Fig. 6). Both the tapetum and retina are well formed. The latter is thrown into numerous large folds.

Fig. 5. Photograph of the rhachischisis posterior.

Labyrinth. The histological structure of the inner ear is too poor to be able to discern the different parts. A large saccus endolymphaticus is present.

(4) *Skeleton*

For the interpretation of the skeletal relations of skull, vertebral column and thorax, it proved necessary to make a wax model.

First of all attention should be paid to a feature, present in all the vertebrae, and also in the skull: they are defective on the dorsal aspect. All the vertebrae are flattened in lateral direction. In the skull this results in the absence of the tectum posterius.

The formula of the vertebral column is normal.

A second abnormal characteristic of the vertebral column consists in a

tendency for doubling in all cervical and most of the thoracic vertebrae. This deformity is due to a splitting in an early stage of the chorda dorsalis, which has led to many complications. Fig. 4 shows that the caudal thoracic vertebrae (Th. 9–12) are complete single vertebrae. All the other cranial vertebrae are

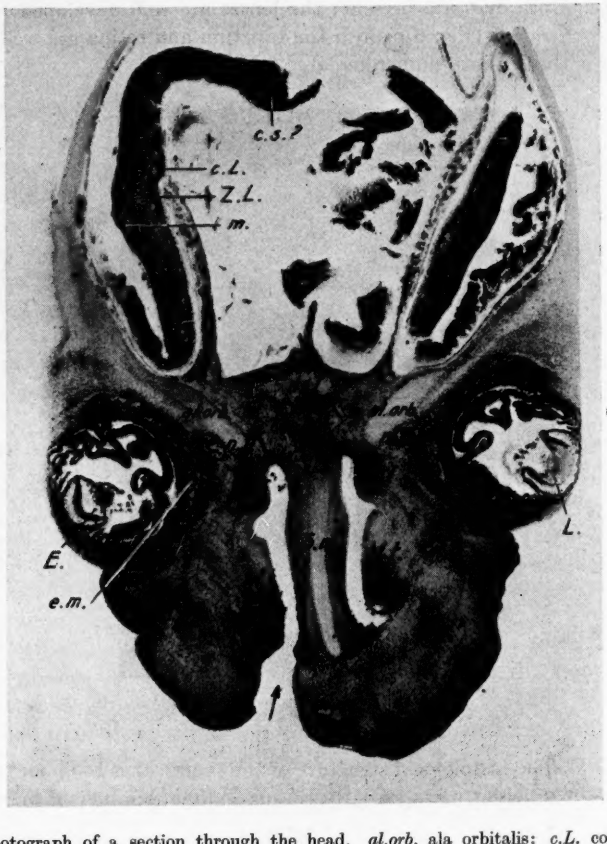

Fig. 6. Photograph of a section through the head. *al.orb.* ala orbitalis; *c.L.* cortex layer; *c.s.* corpus striatum; *E*, eye; *e.m.* eye muscles; *E.t.I*, ethmo-turbinale I; *L*, lens; *m*, matrix; *M.t.* maxillo-turbinale; *S.n.* septum nasi; *Z.L.* intermediate layer.

abnormal. They consist of two *or more* half vertebrae, fused together in an irregular way, e.g. no. 8 consists of four right half vertebrae, and one left half vertebra. The enumeration was made possible by considering the attachment of the ribs and counting the spinal ganglia. It is noteworthy that, in spite of the irregular mode of fusing of the vertebral components, the number of ribs and spinal ganglia, as well as the number of foramina intervertebralia, is normal.

Thorax. The wax model shows that on both sides twelve ribs are present which articulate with the thoracic vertebrae. Of the two longitudinal bars which later form the sternum, the right is connected with the cranial four ribs and the left one with the cranial three ribs. In general the ribs are well developed.

Scapulae and *clavicles* are present and normal in shape.

The *arm skeleton* is normally developed.

Skull. The splanchnocranium as well as the neurocranium are normal, save that the hind part remains open dorsally. No remains of the notochord could be found in the basal part.

(5) *Heart, lungs, intestine and uro-genital organs*

All these organs are present and show no features of special interest. The suprarenal bodies are well developed.

(6) *Trunk musculature*

The trunk musculature seems, when compared with a normal embryo, to be poorly developed.

DISCUSSION[1]

In the introductory section we indicated two categories of malformations of the central nervous system.

The anomaly described in this paper is an example of the first category. The greater part of the central nervous system has disappeared, yet we may conclude from the occurrence of its derivatives (peripheral nerves) that it must have been present in earlier stages.

It seems permissible to make the following generalizations. Cases of "rhachischisis posterior" are not only characterized by a "schisis" of the vertebrae, accompanied by defects of the central nervous system, but also by a disturbance of the vertebral column and dorsal musculature lying underneath these defects. The back region is always shortened as a result of the defective mesodermal inductive mechanism and its defective derivatives, as was experimentally proved in amphibians by Lehmann (1926, 1938). These defective derivatives cause the typical appearance of all these cases. We may conclude that the defective underlying mesoderm is the primary cause of the abnormal development of the central nervous system, which is extroverted and partly open; this, again, is the cause of the non-closure of the neural arches of the vertebrae in the dorsal mid-line.

Quantitatively these defects show great differences. Minor vertebral defects, sometimes of a hereditary character, may not be accompanied by

[1] We herewith express our thanks to Prof. J. J. Th. Vos of the Pathological Department, who kindly put his valuable collection of anencephali at our disposal.

evident abnormalities of the central nervous system. It is interesting to note that in some families individuals occur with vertebral defects, sometimes with and sometimes without accompanying abnormalities of the central nervous system (Schwarzweller, 1937).

SUMMARY

1. Two types of congenital defects of the central nervous system are ascribed to (*a*) a defective underlying inductive mechanism, and (*b*) an abnormal equilibrium of the cerebro-spinal fluid.

2. As an example of the first category the description of a human hemicephalic embryo of 18 mm. is given; the malformation of the central nervous system in this case is due to the abnormal character of the underlying structures.

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THE BLOOD VASCULAR SYSTEM OF THE BRAIN OF *TESTUDO GEOMETRICA*

By G. W. H. SCHEPERS

Department of Anatomy, University of the Witwatersrand, Johannesburg

INTRODUCTORY

In recent years there has been a considerable revival in the study of the comparative features of the blood vessels of the vertebrate brain (Shellshear, 1920, 1929, 1930; Kappers, 1933; Abbie, 1933 (*a* and *b*), 1934; Herrick, 1935; Roofe, 1935). From these studies important information has come to light with regard to doubtful points concerning the structure of the brain; and many problems relating to the evolution of the endocranial vascular system have also been elucidated. In this paper a description is given of the blood vessels of the brain of *Testudo geometrica*. Their comparative features and also their relationship to various landmarks of the brain are discussed. The tortoise brain, however, is comparatively unknown. A brief account of the more striking features of its external morphology has therefore been written and will appear separately. A description of the internal structure of the brain will be published later and the relationship between the blood vascular system and the cell masses and fibre tracts will there be analysed more fully.

The blood vessels of the brain of only one species of tortoise, *T. geometrica*, whose external morphology has already been described and which was obtained from East Bechuanaland, will here be discussed. I wish to thank Mr Pringle of the Port Elizabeth Museum for his assistance in identifying the tortoises. I am also indebted to Mr A. E. Watkins of the Johannesburg Government Mortuary and Medico-Legal Laboratories for procuring me the tortoises which I required.

In order to display the arteries seven specimens were successfully injected. Similar injection preparations were made for the veins, but this was generally not necessary as they were easily demonstrated by constricting the neck of the tortoise immediately after death; the blood was thus retained in the veins and they could be traced to their origin.

When injecting, the following procedure was adopted: the animal was anaesthetized, the ventral shield removed, a canula inserted into the aorta and an incision made into the right auricle. Carmine-gelatine mass was meanwhile warmed in a water-bath until of fluid consistency. A large recording syringe was more suitable for this work than other injecting apparatus which was available. The blood was flushed out with warm normal saline. Next the animal was perfused with 2·5 % formalin for about 5 min. The carmine-gelatine mass was then injected into the aorta, which was thereupon ligatured and the

animal immersed in a bath of iced water. If no great pressure, such as might rupture the vessels, was applied, it was found that the arteries were mostly well injected. Mr I. Macgregor ably assisted me with some of the injection work.

In preparing the illustrations great care was taken to ensure that the average configurations of both the brain itself and the vascular pattern were represented. To attain this accuracy of pictographic representation a micrometer-grid was inserted into the dissecting-microscope ocular and the brain and blood vessels were then drawn to scale on graph paper.

The sketches made in this manner were carefully compared with others prepared from serially sectioned brains by the cartographic method ordinarily used in this department for mapping cell masses of the brain (Dart, 1934). To secure natural contours, in the case of these reconstructions, modifications of this technique, which are described in the account of the external morphology of the brain, were used.

I am indebted to Prof. R. A. Dart for entrusting this investigation to me, for the facilities provided in his department and for advice given at various stages of the progress of the work. I wish to extend my sincere thanks also to Prof. C. J. van der Horst and to Dr J. Gillman for many welcome suggestions.

DESCRIPTION

The internal carotid vessels are solely responsible for the arterial blood supply, as there are no vertebral arteries in this brain.

From the ventral and the lateral aspects of the brain (Figs. 2 and 3) each internal carotid artery may be seen to pass forward and upward, against the side of the hypothalamus, to its bifurcation into cranial and caudal divisions, opposite the posterior margin of the optic tract.

Before this bifurcation the carotid constantly gives off two small branches. The larger passes forward, proceeding along the infero-lateral aspect of the optic nerve; this is the *ophthalmic artery* (Kappers, 1933). The other arises opposite this ophthalmic and is directed backward and downward, ramifying on the hypothalamus and giving a few small branches to the hypophysis; Abbie (1934) named it the "hypophyseal artery", but *hypothalamic artery* may be a better name.

The cranial and caudal divisions of the carotid pass horizontally forward and backward respectively (Fig. 8). But both may also course a little upward in some cases.

The Cranial Division of the Carotid

As has been frequently recognized (Kappers, Hofmann, Abbie, De Vriese, etc.), not only the mammalian, but also the reptilian forebrain derives its blood supply from three large blood vessels, two of which (the anterior and posterior cerebral arteries) are distributed over the medial surface, and the third and largest (the middle cerebral artery) over the lateral aspect. In *T. geometrica* all

three of these vessels are derived from the cranial division of the carotid in about 80 % of cases (Figs. 2 and 3). Otherwise the posterior cerebral arises from the caudal division, after the manner indicated (Fig. 2) as an alternative

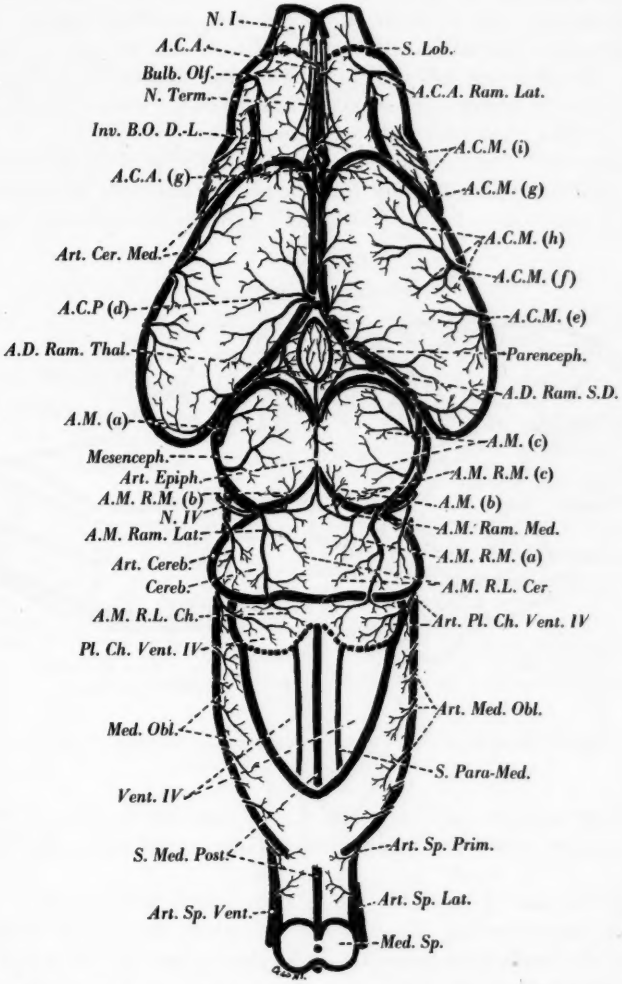

Fig. 1. The arterial supply of the dorsal aspect of the brain of *Testudo geometrica*. (10 × natural size.)

mode of origin. When the latter condition obtains the cranial division of the carotid passes forward to bifurcate rather abruptly into the anterior and middle cerebral arteries.

The anterior cerebral artery

Though appearing to be a continuation of the cranial division of the carotid, the anterior cerebral artery is smaller than the middle cerebral artery. It continues forward and medially across the diagonal band region. Here it usually gives off a medial branch (Figs. 3 and 4, *A.C.A.* (*a*)) which is continued on to the medial aspect and runs upward in the rudimentary sulcus primus. The vessel partly supplies the diagonal band, and its terminal ramifications reach the lamina terminalis and the commissures.

The anterior cerebral extends directly forward after this. It passes either over the summit of the olfactory tubercle or, if it has come to lie sufficiently medially it runs forward internally to this tubercle and thus lies near to its

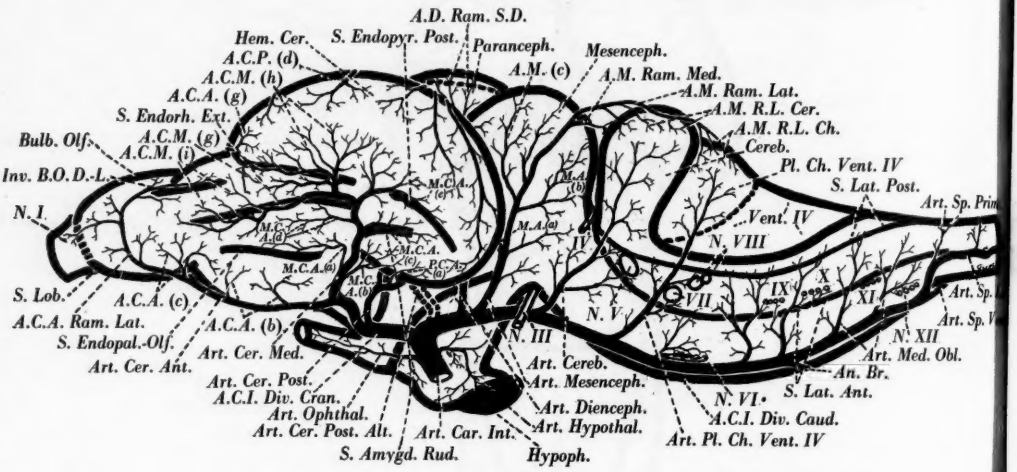

Fig. 2. The arterial supply of the lateral aspect of the brain of *Testudo geometrica*. (10 × natural size.)

fellow of the opposite side, as in Fig. 3. On reaching the coronal sulcus the artery appears to end by dividing into a large medial and a smaller lateral branch.

Along this part of its course two or more lateral branches (Figs. 2 and 3, *A.C.A.* (*b*)) are given off. These supply the whole area of the infero-lateral aspect of the hemisphere below the endopalaeo-olfactory sulcus and in front of the endorhinal sulcus.

The lateral terminal branch arising at the coronal sulcus passes forward on the inferior surface of the olfactory bulb, remaining more or less near the mid-line (Fig. 3, *A.C.A. Ram. Lat.*). Just caudally to the sulcus lobaris it turns abruptly laterally and dorsally (Fig. 2). On reaching the dorso-lateral aspect of the bulb the vessel again turns almost horizontally backward, runs in the groove

corresponding to the dorso-lateral invagination of the bulb and terminates near the coronal sulcus (Figs. 1 and 2). Three sets of terminal branches are given off. One arises near its commencement. Others arise along its course. The third

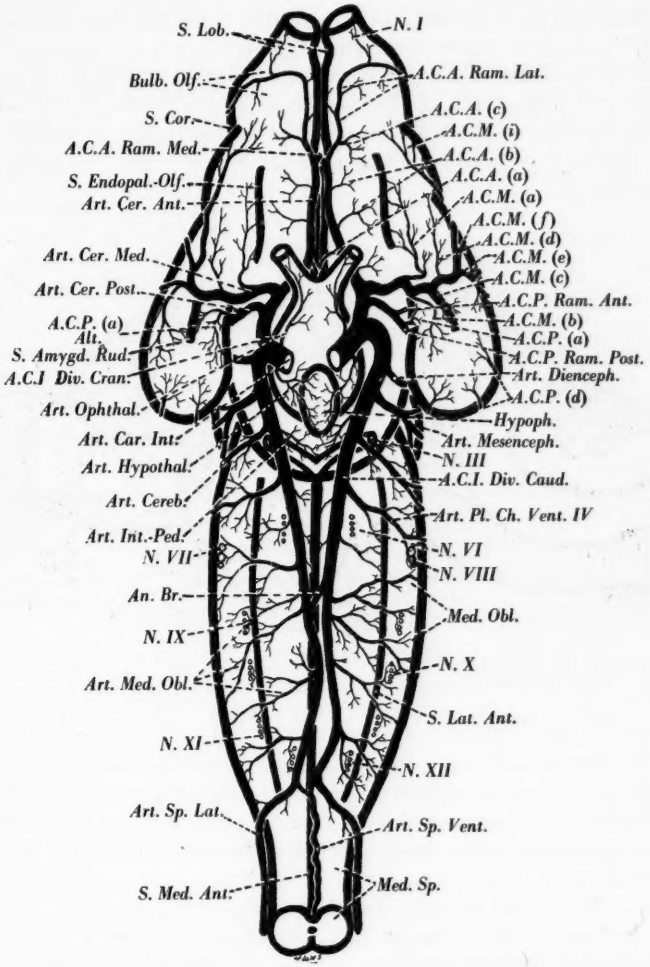

Fig. 3. The arterial supply of the ventral aspect of the brain of *Testudo geometrica*. (10 × natural size.)

group consists of branches arising opposite the dorso-lateral invagination and passing into it. This lateral branch thus supplies the inferior and lateral aspects and the outer half of the dorsal aspect of the olfactory bulb.

The large medial branch appears to be the continuation of the artery. It passes obliquely forward and upward, entering the interval between the olfactory bulbs opposite the coronal sulcus. Its further course may now be traced on the medial surface of the brain (Fig. 4). Passing obliquely upward and forward and then upward and backward to reach the upper border of the olfactory bulb at the sulcus coronalis, it describes a curve which is convex forward. It lies superficially to the nervus terminalis. Branches are given off from this part (Fig. 4, *A.C.A.* (*d*)), and these supply practically the whole of the medial surface of the bulb and also the medial half of its dorsal surface (Fig. 1). There is generally one larger branch which passes forward on to the olfactory nerve.

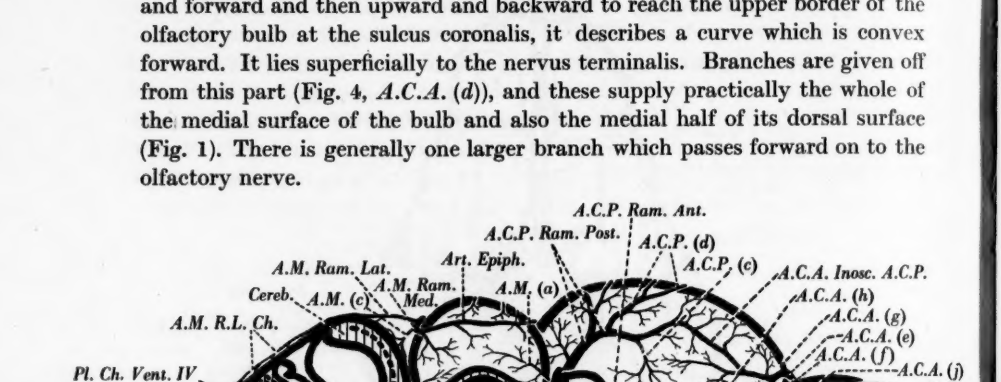

Fig. 4. The arterial supply of the medial aspect of the brain of *Testudo geometrica*. (10 × natural size.)

At the coronal sulcus and thus opposite the proximal end of the terminal nerve, the medial continuation of the anterior cerebral artery bifurcates forming inferior (*A.C.A.* (*f*)) and superior (*A.C.A.* (*e*)) branches of about equal size. The superior branch passes directly across the coronal sulcus and, just below the anterior pole of the hemisphere, it subdivides into medial and lateral branches. The lateral branch (*A.C.A.* (*g*), Figs. 1, 2 and 3) is much the smaller and passes directly upward and backward over the medial aspect of the anterior pole of the hemisphere. As could be determined histologically, it has no relation whatsoever to the pyriform cortex, being restricted to the part of the hippocampal formation also lodged in this pole. The medial division continues almost horizontally backward, against the medial surface of the hemisphere, for a short distance. It then gives off a large but short descending branch (*i*), which anastomoses with the inferior terminal branch (*A.C.A.* (*f*)), where it leaves the coronal sulcus. Beyond this

anastomotic trunk the artery turns upward and backward and approximately at the junction of the anterior fourth and posterior three-fourths of the medial surface of the hemisphere, it becomes continuous with the posterior cerebral artery by direct inosculation. It gives off branches all along its course to the upper two-thirds of the medial surface, above the level of the interventricular foramen.

The inferior branch (Fig. 4, *A.C.A.* (*f*)) courses downward and backward along the coronal sulcus, soon receiving the anastomotic branch (*i*) from the medial branch of the superior division (*A.C.A.* (*e*)). It then divides into horizontal and descending branches. The latter (*A.C.A.* (*j*)) follows the coronal sulcus and gives off small twigs which supply part of the area of the medial surface of the olfactory bulb enclosed between it and the medial continuation of the anterior cerebral artery. It leaves the coronal sulcus at the septo-striate sulcus to follow this more or less for a short distance, terminating over the area of the medial surface below this latter sulcus and in front of the sulcus primus.

The horizontal branch runs backward slightly above the septo-cortical sulcus for a short distance. Then it subdivides into a continuing branch (*A.C.A.* (*h*)), which is distributed over the lower third of the medial surface as far posteriorly as the interventricular foramen, and a descending branch (*k*). The latter is distributed over the septal region (branches (*j*)), an area which is bounded below by the septo-striate sulcus and above by the septo-cortical sulcus. It contributes also to the vascular supply of the lamina supra-neuroporica, the commissures (partly), the roof of the dorsal recess of the third ventricle, and the choroid plexus of the lateral ventricle, reaching these structures by a small branch which runs along the septo-cortical sulcus.

Summary of the branches of the anterior cerebral artery

(1) *From the main stem:*

- (*a*) Small medial branch to diagonal band, lamina terminalis and commissures (*A.C.A.* (*a*)).
- (*b*) Two or more lateral branches to tuberculum olfactorium (*A.C.A.* (*b* and *c*)).
- (*c*) Lateral division to lateral aspect of olfactory bulb (*A.C.A. Ram. Lat.*).
  - (i) Small lateral branches.
  - (ii) Numerous terminal branches.
  - (iii) Branches to dorso-lateral invagination of bulb.
- (*d*) Medial division to medial aspect of olfactory bulb and hemisphere (*A.C.A. Ram. Med.*).

(2) *Terminal branches of:*

- (*a*) *Inferior terminal branch* (*A.C.A.* (*f*)).
  - (i) *Descending branch* to the part of the tuberculum olfactorium and striate region extending on to the medial surface (*A.C.A.* (*j*)).

(ii) *Horizontal branch.*

(1) Continuing branch to lower third of hippocampal area as far as interventricular foramen (*A.C.A.* (*h*)).

(2) Descending or septal branch to septal area, lamina supraneuroporica, commissures and choroid plexus of lateral ventricle ((*k*) and (*j*)).

(*b*) *Superior terminal branch* (*A.C.A.* (*e*)).

(i) *Lateral branch* to medial surface of anterior pole (*A.C.A.* (*g*)).

(ii) *Medial branch.*

(1) To anterior fourth of medial surface of hippocampus.

(2) To inosculate with posterior cerebral.

(3) Anastomotic with inferior terminal branch (*i*).

The middle cerebral artery

This is the most cranial of the large blood vessels primarily distributed over the lateral aspect of the brain for it supplies the greater part of the lateral aspect of the cerebral hemisphere. From its origin it is directed upward and outward, generally also slightly backward, to reach the sulcus endorhinalis, to the floor of which it is applied for a great part of its further course (*vide* Fig. 2).

The middle cerebral artery has a very tortuous course, and two main sharp backward convexities and one directed forward between these two, can generally be recognized. The lower backward convexity is related to the sulcus representing the rudimentary amygdaloid fissure. The forward convexity is usually quite sharp and may be directed slightly downward as well. The upper backward convexity is blunter. From here the generally tortuous terminal portion of the artery runs almost horizontally forward, still following the endorhinal sulcus, to terminate anteriorly at the level of the coronal sulcus.

There are numerous large branches by means of which an extensive portion of the lateral wall of the brain is supplied. These are the following: From the stem of the vessel two small branches may usually be found to arise. *A.C.M.* (*a*) passes anteriorly and upward and then forward in the endotubercular sulcus, and is distributed over the upper part of the tuberculum olfactorium posteriorly. Branch *A.C.M.* (*b*) which arises approximately opposite this latter branch, is directed backward and upward, so that it accompanies the middle cerebral artery for a short distance. It is distributed over the area immediately below the rudimentary amygdaloid sulcus. It also partly supplies the optic tract. This branch, whose area of supply is restricted to this more or less circumscribed region, seems to be a fairly constant feature of the vascular pattern. It may, however, occasionally be absent, in which case other vessels, branches of the posterior cerebral artery, take its place. It may also arise independently from the cranial division of the carotid. *A.C.M.* (*c*) is a branch which arises at the apex of the lower backward convexity and runs backward

along the rudimentary amygdaloid sulcus, to the floor of which it is firmly secured by means of large penetrating vessels. It terminates over the adjacent areas. *A.C.M.* (*d*) arises from the apex of the forward convexity as a small branch, which, together with *A.C.M.* (*a*) supplies the part of the tuberculum olfactorium and striatal region above the endotubercular sulcus. From the upper backward convexity or more often from the lower convexity (Fig. 2) a large branch (*A.C.M.* (*e*)) arises which passes upward and backward over the pyriform lobe, following the course of the posterior endopyriform sulcus and supplying a large portion of the lateral and dorso-lateral surfaces of the hemisphere (Figs. 1 and 2).

At the upper backward convexity of the middle cerebral vessel a large branch (*A.C.M.* (*f*)) usually arises. It runs upward and forward towards the anterior endopyriform sulcus in which it terminates eventually. Between the endorhinal and the endopyriform sulci a large branch (*A.C.M.* (*g*)) is given off, and it supplies the area intervening between the two sulci. Large branches (*A.C.M.* (*h*)) arise along its course in the endopyriform sulcus and they distribute over the outer half of the anterior third of the dorso-lateral surface of the hemisphere above the level of the endopyriform sulcus. Sometimes this branch (*A.C.M.* (*f*)) is replaced by a number of large vessels which pass vertically upward from the middle cerebral artery as it lies in the endorhinal sulcus.

The middle cerebral artery terminates (Fig. 2) after continuing forward in the endorhinal sulcus, by giving off numerous branches (*A.C.M.* (*i*)) to the bulging area bounded in front by the coronal sulcus, below by the endorhinal sulcus and above by the endopyriform sulcus. These branches can be seen also in Figs. 1 and 3.

All along their course the middle cerebral artery and its branches give off perforating branches which enter the lateral wall of the hemisphere and anchor this artery so firmly to its surface that it is impossible to move the vessel as a whole without severing these penetrating branches. The largest branches occur in the endorhinal sulcus where the fovea is deepest. Others are present all along the anterior and posterior endopyriform sulci and also the rudimentary amygdaloid sulcus. These vessels are the important "striate" and "pyriform" arteries (Abbie, 1934). As their distribution on the one hand depends on internal arrangement of the pyriform cell mass derivatives and on the other hand determines the arrangement of the middle cerebral artery and its branches, the latter has in *T. geometrica* a fairly constant and characteristic pattern of distribution.

Summary of the branches of the middle cerebral artery

(1) *Anterior branches:*

(*a*) a branch from the stem (*A.C.M.* (*a*));

(*b*) a branch from the anterior convexity (*A.C.M.* (*d*)).

These supply the tuberculum olfactorium and striatum above the endo-palaeo-olfactory sulcus.

(2) *Posterior branches:*

(*a*) a branch to the region below the amygdaloid sulcus (*A.C.M.* (*b*));
(*b*) a branch along the amygdaloid sulcus (*A.C.M.* (*e*));
(*c*) a branch along the posterior endopyriform sulcus (*A.C.M.* (*e*));
(*d*) a branch along the anterior endopyriform sulcus (*A.C.M.* (*f*)).

(3) *Terminal branches:*

(*a*) branch *A.C.M.* (*f*) gives rise to *A.C.M.* (*g*) and *A.C.M.* (*h*) to regions respectively above and below it;
(*b*) middle cerebral artery ends near coronal sulcus in branches *A.C.M.* (*i*).

The posterior cerebral artery

This artery, when it arises from the anterior division of the carotid, passes outward, upward and backward obliquely across the optic tract and disappears behind the posterior pole of the hemisphere opposite the middle of this tract. If the artery is derived from the posterior division of the carotid it passes directly laterally and upward to disappear behind the posterior pole of the hemisphere at its inferior margin. Both these modes of origin are illustrated in Figs. 2 and 3. Its further course can be traced on the medial aspect of the hemisphere. Before reaching this surface, as is seen in Figs. 2 and 3, or immediately after doing so, it bifurcates, forming anterior and posterior divisions.

The posterior division (*A.C.P. Ram. Post.*) which is large and is more or less a direct continuation of the artery, runs upward and backward on the medial surface of the hemisphere to appear, in Fig. 4, opposite the habenula. During this part of its course it lies approximately midway between the periphery of the medial surface of the hemisphere and the interventricular foramen. Beyond this point it courses forward in an upward convex arch which, though tortuous throughout, follows the endohippocampal sulcus closely and may thus be seen along the line of junction of the upper third and lower two-thirds of the part of the medial surface of the hemisphere above the interventricular foramen and septo-cortical sulcus. Anteriorly it anastomoses directly with the medial branch of the superior terminal division of the anterior cerebral, as mentioned above.

Branches arise from this artery all along its course, to supply the adjacent areas of brain surface. At its commencement a small twig (*A.C.P.* (*a*)) may usually be found to supply the inferior surface of the posterior pole of the hemisphere (Figs. 2 and 3). When *A.C.M.* (*b*) is absent this branch enlarges to supply the whole amygdaloid region as in Fig. 3. Along its vertical portion the branches are small, so that those passing peripherally do not quite reach the external surface. Along its horizontal portion, however, they are large and more numerous and appear on the dorsal and lateral aspects (Figs. 1 and 2, *A.C.P.* (*d*)). The branches from this portion are distinguished from those of the anterior cerebral by differences in their slope. They pass obliquely *forward*, upward and downward, whereas those of the anterior cerebral pass obliquely

backward, upward or downward. A large branch arises from the artery at the junction of its horizontal and vertical portions.

On reaching the dorso-lateral surface, where they terminate, these branches distribute blood anteriorly to the medial third and posteriorly to the medial half of the posterior four-fifths of this surface (Figs. 1 and 2).

The area between the vertical part of the artery and the interventricular foramen is supplied, in part, by small centripetal branches (*A.C.P.* (*b*)) from the vertical part. Some of these may contribute to the formation of the choroid plexus of the lateral ventricle. The remainder of the medial surface visible in Fig. 4 is supplied by a large branch (*A.C.P.* (*c*)) passing ventrally from the horizontal part of the artery.

The *anterior division* of the posterior cerebral artery (*A.C.P. Ram. Ant.*) is directed obliquely forward and upward on the medial surface of the hemisphere, until it is just above the level of the interventricular foramen. Then it turns downward and supplies the choroid plexus along its line of invagination into the lateral ventricle through the interventricular foramen. It is joined, in doing so, by the branches from the vertical limb of the posterior division of the posterior cerebral and the branch from the anterior cerebral artery which approaches the foramen along the septo-cortical sulcus. All along its course arise branches, which supply portions of the medial surface on either side of the artery.

It is doubtful whether any arterial supply to the dorsal sac or parencephalon and the choroid plexus of the third ventricle is derived from the posterior cerebral artery. At least no such vessels could be demonstrated.

Summary of the branches of the posterior cerebral artery

(1) *Posterior division* (*A.C.P. Ram. Post.*):

- (*a*) A branch to the inferior surface of the posterior pole of the hemisphere (*A.C.P.* (*a*)).
- (*b*) A central branch (*A.C.P.* (*b*)) which supplies:
  - (i) the lower two-thirds of medial surface of posterior pole;
  - (ii) the choroid plexus of lateral ventricle.
- (*c*) A central branch to lower two-thirds of central third of medial surface (*A.C.P.* (*c*)).
- (*d*) Peripheral branches (*A.C.P.* (*d*)), which supply:
  - (i) the upper third of the posterior four-fifths of the medial surface of the hemisphere;
  - (ii) the medial third anteriorly and the medial half posteriorly of the posterior four-fifths of the dorso-lateral surface of the hemisphere.

(2) *Anterior division* (*A.C.P. Ram. Ant.*):

- (*a*) Branches to areas adjacent to it.
- (*b*) Branches to the choroid plexus of the lateral ventricle.

The Caudal Division of the Carotid

This division of the carotid may pass either horizontally backward and medially from the bifurcation, or may pass obliquely upward. The caudal divisions from the two sides gradually approximate to one another. As each caudal division passes backward it bridges across the notch between the cerebral peduncle and the hypothalamus and while doing this lies ventrally to the oculomotor nerve. The two nerves thus embrace the two arteries. Just anteriorly to these two nerves an anastomotic bridge, lying on the posterior aspect of the hypothalamus, may sometimes connect the two caudal divisions (Figs. 2 and 3, *An. Br.*). These blood vessels run backward on the ventral surface of the medulla oblongata and eventually come to lie close to one another. Anastomotic bridges may then occur anywhere along this section or no bridge may occur. At the posterior limit of the medulla oblongata the two arteries may join to form a single small median vessel which follows a tortuous course along the ventral midline of the spinal medulla. When no such union takes place branches from the posterior divisions form this median blood vessel. This median artery is the ventral spinal artery described by Hofmann (1900) as occurring in most vertebrates. At the posterior end of the medulla oblongata two conspicuous lateral spinal arteries continue for some distance. In no instance, however, were "vertebral arteries" displayed.

All along the course of this caudal division of the carotid lateral branches are given off, each of which has a definite region of distribution which is constant for this series of brains. In progression backward these correspond to (i) the diencephalon and dorsal sac, (ii) the mesencephalon, (iii) the cerebellum, (iv) the choroid plexus of the fourth ventricle, (v) pseudo-segmental vessels to the cranial nerve roots and the medulla oblongata, (vi) the last branch, which arises from the lateral spinal artery, runs laterally in the groove which defines the medulla oblongata from the spinal medulla. It is the first spinal artery. A medial vessel is given off by each caudal vessel as well, supplying the interpeduncular region.

The diencephalic artery

The diencephalic artery arises immediately behind the posterior cerebral artery, when this is a branch of the caudal division. This origin is almost invariably opposite the posterior margin of the optic tract. From here the artery passes directly upward and laterally and disappears like the posterior cerebral artery behind the posterior pole of the hemisphere. Sometimes (but on the whole rarely) it occurs as a branch of the posterior cerebral artery, arising from it along the lower end of the vertical part of its course against the medial surface of the hemisphere. In both cases it may be traced beyond this point as it passes upward against the lateral surface of the thalamus. It very soon divides into two, viz. a larger anterior or thalamic branch and a posterior and more lateral branch to the dorsal sac and its choroid plexus.

The *thalamic branch proper* (*A.D. Ram. Thal.*) subdivides into a series of smaller branches which are distributed over the whole of the external surface of the thalamic region. Some of these enter the lesser choroid plexus of the third ventricle and dorsal sac, which is related in this brain to the lower surface of the anterior epiphysial or parapineal body (Schepers, 1938).

The *branch to the dorsal sac* (*A.D. Ram. S.D.*) ascends on the external surface of the sac, subdividing here into smaller branches which reach the dorsal surface of the sac and descend into its dorsal depression. These branches may supply the epiphysial bodies to some extent, but most of the branches pass into the choroid plexus of the third ventricle, which invaginate here in the angle between the lateral walls and the floor of the depression (Figs. 1 and 2).

The mesencephalic artery

This artery (Fig. 2) is larger than the diencephalic artery. It arises behind the latter and shortly anteriorly to the oculomotor nerve, and is directed upward and backward, pursuing an almost straight course for nearly the whole of its length. It crosses only part of the optic tract, and reaches the middle of the lateral aspect of the optic lobe. From here its terminal portion continues upward and backward and appears eventually on the dorsal aspect of the lobe (Fig. 1). Opposite the posterior margin of this lobe it terminates by dividing into two branches, viz. a medial and a lateral.

The *medial terminal branch* (*A.M. Ram. Med.*), passing directly inward to the midline, gives off branches to the upper part of the central portion of the anterior convex surface of the cerebellar fold (*A.M.R.M.* (*a*)), to the posterior half of the medial surface of the optic lobe (*A.M.R.M.* (*b*)), and finally terminates by anastomosing with that of the opposite side, to form a median artery (*A.M.R.M.* (*c*)) which passes forward, deep to the dorsal venous sinus, described later, to the two epiphysial structures embedded in the roof of the dorsal sac, which it supplies.

The *lateral terminal branch* (*A.M. Ram. Lat.*) continues posteriorly over the cerebellum, supplying together with that of the opposite side the remainder of its dorsal surface (*A.M.R.L. Cer.*). It ends by ramification over the upper surface of the masses of choroid tissue in the fourth ventricle (*A.M.R.L. Ch.*).

Three of the branches from the stem of the mesencephalic artery are constantly present on the lateral surface of the optic lobe. One arises from it shortly after it crosses the inferior margin of the optic tract (Fig. 2, *A.M.* (*a*)). It is large, and, passing upward and forward, supplies approximately the anterior half of the lobe, some of its ramifications reaching the medial aspect by passing round the anterior end of the lobe. The other two branches (*A.M.* (*b* and *c*)), together with smaller less constant twigs and the terminal branches of the artery, are distributed over the posterior halves of the lobes. One of these branches (*A.M.* (*b*)) passes directly backward round the base of the optic lobes and disappears in the interval between the optic lobe and the cerebellar

fold. It supplies branches to the adjacent regions of the optic lobe and to the lower portion of the intermediate third of the anterior surface of the cerebellum. The other branch (*A.M.* (*c*)), generally accompanied by a smaller one closely similar to it, arises shortly after the previous branch, and, passing vertically upward, ramifies over the posterior two-thirds of the dorsal surface of the optic lobe.

Summary of the branches of the mesencephalic artery

(1) *Branches from the stem:*

- (*a*) Anterior branch to anterior half of lobe (*A.M.* (*a*)).
- (*b*) Posterior horizontal branch to optic lobe and base of cerebellum (*A.M.* (*b*)).
- (*c*) Ascending branches to dorsal surface of posterior half of the optic lobe (*A.M.* (*c*)).

(2) *Terminal branches:*

- (i) Medial (*A.M. Ram. Med.*)
  - (*a*) to cerebellum: intermediate third of anterior surface (*A.M.R.M.* (*a*));
  - (*b*) to optic lobes (*A.M.R.M.* (*b*));
  - (*c*) to epiphysial bodies in conjunction with that of opposite side (*A.M.R.M.* (*c*)).
- (ii) Lateral (*A.M. Ram. Lat.*)
  - (*a*) to cerebellum: lateral thirds of dorsal surface (*A.M.R.L. Cer.*);
  - (*b*) to choroid plexus of fourth ventricle (*A.M.R.L. Ch.*).

The cerebellar artery

This artery is slightly smaller than the preceding one, but resembles it closely. It arises from the caudal division of the carotid, just behind the oculomotor nerve, and coursing outward, upward and backward against the lateral surface of the cerebral peduncles, reaches the antero-lateral surfaces of the cerebellum, passing either anteriorly or posteriorly to the trochlear nerve, as this emerges between the bases of the cerebellum and the tectum. The artery terminates by ramifying over the external surface of the outer third of the cerebellum. Lower down, immediately adjacent to its main stem, it supplies areas of the cerebral peduncles. These branches, however, produce no constant pattern.

The artery to the choroid plexus of fourth ventricle

This artery arises from the caudal division of the carotid in the same way as the preceding one. The interval between the two is about equal to that between the cerebellar and mesencephalic branches. The artery courses upward just posteriorly to the root of the trigeminal nerve, being parallel to the cerebellar artery all the time. It terminates in the choroid plexus of the fourth

ventricle, where it may also anastomose with twigs from the lateral terminal division of the mesencephalic artery. In its course against the medulla oblongata it gives off conspicuous branches to it, especially in the region of the sixth nerve.

The pseudo-segmental arteries to the medulla oblongata

Arising from the terminal portion of the posterior division of the carotid are five small branches which seem to be arranged segmentally. They are spaced at approximately equal distances from one another, the last one arising from the lateral spinal branch at the region of junction of the spinal cord and the medulla oblongata, being the first spinal artery. The other four seem to be related to the roots of the last cranial nerves.

The interpeduncular artery

Lastly should be mentioned the small arterial twig passing medially from the caudal division of the carotid between the origins of the mesencephalic and cerebellar arteries and running along the oculomotor nerve, to terminate in the interval between the two "cerebral peduncles" in the region which corresponds to the posterior perforated substance in the mammalian brain.

The essential pattern of the arterial vascular system of the reptilian brain, as described above, is, that laterally large branches and medially smaller branches arise from the longitudinal axis, situated at the base of the brain on each side and deriving its supply of blood from the carotid vessels. The most striking feature of this vascular system is that apparently every major morphological unit of the brain has its own arterial trunk, almost as if the branches are distributed to "segments" of the brain. The size, the form and the degree of complication of the ramifications of each artery seem to depend directly on the extent and arrangement of the brain areas supplied. Consequently, in this species, such brain areas are accurately demarcated by the branches and subdivisions of the arterial system.

The areas of supply can be summarized as follows: On the ***cerebral hemispheres:*** (i) upper and lower halves of the medial and the lateral surfaces of the *olfactory* bulbs (Figs. 1–4) supplied by corresponding branches of the anterior cerebral artery; (ii) the *septal region*, *lamina supraneuroporica* and *dorsal commissure* (Fig. 4) supplied by horizontal branches from the inferior terminal division of the anterior cerebral artery; (iii) the *diagonal band region*, the *lamina terminalis* and the *ventral commissure* (Figs. 3 and 4), supplied by the medial branch from the first portion of the anterior cerebral artery; (iv) the *striatal region* of the medial aspect and lower part of the medial surface of the olfactory bulb (Fig. 4), supplied by the descending branch of the inferior terminal division of the anterior cerebral artery; (v) the *anterior third* of the *upper half* of the *medial surface* (Fig. 4), and the corresponding *medial third* of the *dorsal surface* of the anterior fifth of the hemisphere (Fig. 1), supplied by the terminal branches of the anterior cerebral artery; (vi) the remainder of

the *medial surface* or the *upper* and the *lower zone* (Fig. 4), and the *medial half* of the *posterior four-fifths* of the *dorsal surface* (Figs. 1 and 2) supplied by the anterior and the posterior divisions of the posterior cerebral artery; (vii) the *tuberculum olfactorium* and the *striate region* (Fig. 3), directly supplied by the anterior cerebral artery in its course over these; (viii) the *amygdaloid region* (Figs. 2 and 3), supplied by branches from the middle cerebral artery and the anterior division of the posterior cerebral arteries; (ix) the *remainder* of the *lateral wall* of the hemisphere by the middle cerebral artery. This latter region is again subdivisible into many distinct regions, viz.: an area between the endo-palaeo-olfactory sulcus and the endorhinal sulcus, an area between the latter sulcus and the anterior endopyriform sulcus, an area above this latter sulcus, and areas adjacent to the posterior endopyriform sulcus (Fig. 2).

In the ***diencephalon*** the *hypothalamus* is supplied separately (Figs. 2 and 3). The *thalamus* on the one hand, and the *epithalamic region* and the *dorsal sac* on the other hand are supplied by the anterior and posterior branches respectively of the diencephalic artery.

Each half of the ***tectum of the mesencephalon***, in accordance with its blood supply from the branches of the mesencephalic artery, can be mapped out into *anterior* and *posterior regions*.

The *central third* of the ***cerebellum***, in which distinct *upper* and *lower halves* may be recognized, is supplied by the terminal branches of the mesencephalic artery. The *lateral thirds* are supplied by the cerebellar arteries.

The artery of supply to the ***choroid plexus*** of the fourth ventricle supplies also the *region of the trigeminal nerve*. The ***medulla oblongata*** is supplied *pseudo-segmentally* from arteries corresponding to the last four cranial nerves. There is a special arterial supply to the *interpeduncular region*.

It is evident from the foregoing, that each major structure in the brain not only has a constant arterial supply, but that various localized areas in those regions, where great expansion has taken place, have also acquired a special system of supply by means of arteries, which are constant features of the vascular system of the *Testudo geometrica* species. A relatively constant arterial vascular pattern is thus established.

The venous system

While the arterial supply of the brain is derived from the ventral aspect, via the internal carotid artery and its branches, the venous drainage is apparently entirely towards the dorsal surface. Curiously too, as regards its main features, the pattern of venous drainage is subject to very little variation, even less than occurs ordinarily in the case of the arterial pattern. This is contrary to the condition in *Amblystoma* (Roofe, 1935).

The termination of the venous trunks of the brain is in a large dorsal longitudinal vein, namely: (i) the olfactory veins, (ii) the medial cerebral veins, (iii) the common choroidal vein from the choroid plexus of the lateral ventricles,

(iv) the dorso-medial cerebral veins and their branches, (v) the posterior lateral cerebral vein. Into the dorsal longitudinal vein large vessels also drain directly

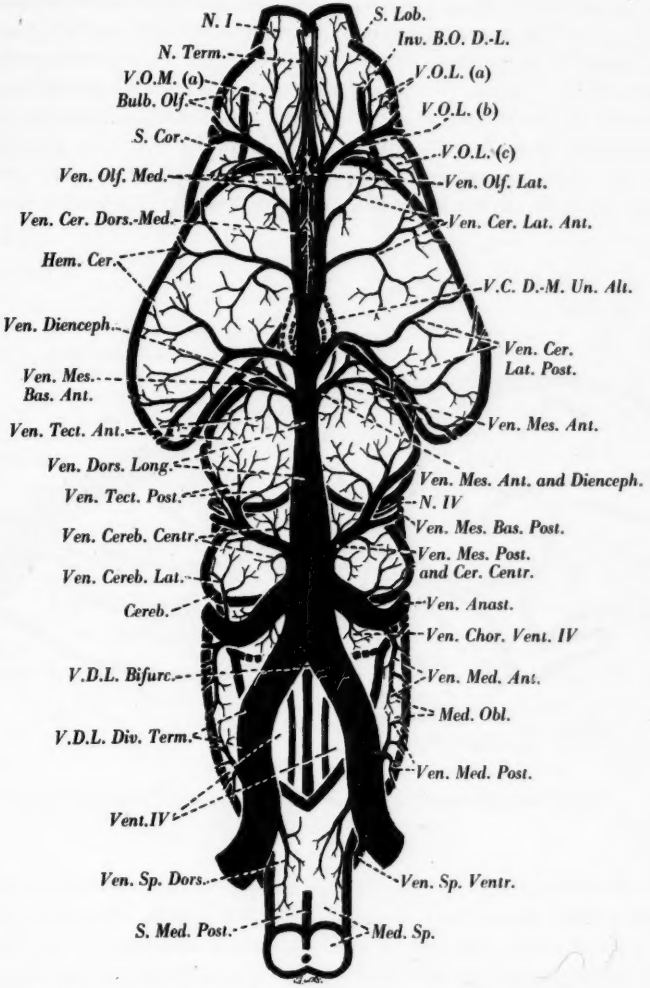

Fig. 5. The venous drainage of the dorsal aspect of the brain of *Testudo geometrica*. (10 × natural size.)

from the mesencephalon, the cerebellum, the medulla oblongata and the choroid plexus of the fourth ventricle.

The olfactory veins

These come from both the medial and the lateral surfaces of the olfactory bulbs. The lateral surface affords a greater area, the more so on account of the presence in it of the dorso-lateral bulbar invagination. The *lateral olfactory vein* (Figs. 5, 6 and 7) is therefore correspondingly larger. Its numerous tributaries converge in three groups (***V.O.L.*** (*a*)). The most anterior set drains practically the whole of the dorso-lateral and the dorsal surfaces of the olfactory bulb. It also drains the dorso-lateral bulbar invagination (***V.O.L.*** (*b*)). The next and largest branch is formed by tributaries from (i) the ventro-lateral surface of the olfactory bulb, (ii) the lower half of the tuberculum olfactorium and striate

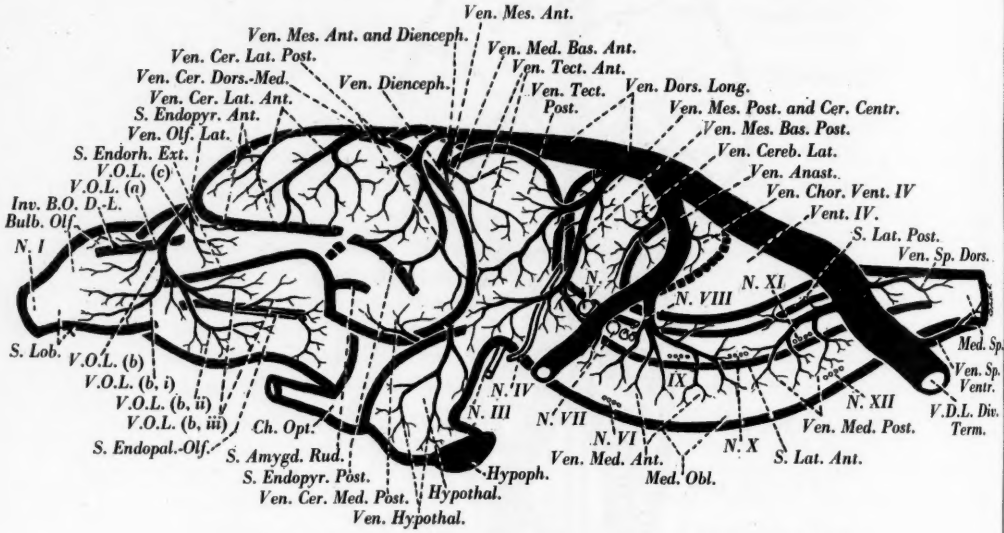

Fig. 6. The venous drainage of the lateral aspect of the brain of *Testudo geometrica*. (10 × natural size.)

region of the lateral aspect, and (iii) the remainder of the tuberculum olfactorium and the striate above the endopalaeo-olfactory sulcus. Branches (*a*) and (*b*) form a single vessel coursing upward, backward and medially on the dorso-lateral surface at the posterior end of the dorso-lateral bulbar invagination. This vessel is joined opposite the anterior pole of the hemisphere by the third branch (***V.O.L.*** (*c*)) of the lateral olfactory vein. This branch drains the area of the lateral surface of the olfactory lobe of the hemisphere between the endorhinal and the anterior endopyriform sulci.

The *medial olfactory vein* (Figs. 5 and 8) has two main tributaries (***V.O.M.*** (*a*)). A small vessel, seen best from above, drains the medial half of the dorsal surface

of the olfactory bulb (*V.O.M.* (*b*)). This branch drains the medial aspect of the olfactory bulb as far posteriorly as the coronal sulcus and is joined by branch (*a*) to form the medial olfactory vein.

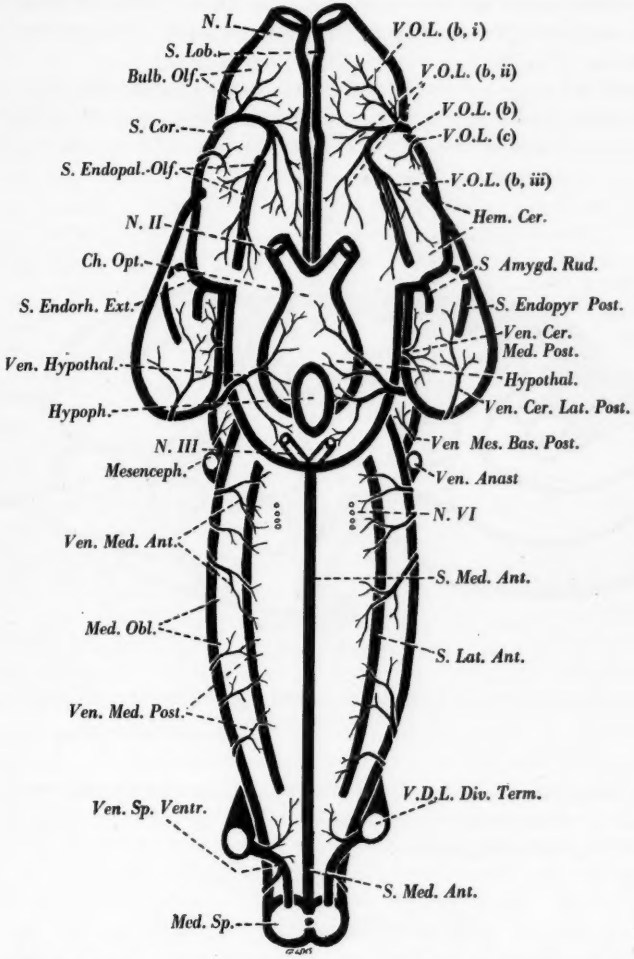

Fig. 7. The venous drainage of the ventral aspect of the brain of *Testudo geometrica*. (10 × natural size.)

The dorso-medial cerebral veins

The lateral and the medial olfactory veins unite at the medial side of the anterior pole of the hemisphere and form the dorso-medial cerebral veins on each side (Figs. 5 and 8). These are applied to the dorso-medial borders of the

hemispheres, while these are in contact with one another. Where these borders diverge to embrace the dorsal sac, the dorso-median cerebral veins unite to form a single vessel, which is joined by the median ventral vessels and is the commencement of the dorsal longitudinal vein. In rare instances these cerebral veins do not unite in this fashion, and then they join the posterior cerebral veins instead (Fig. 5).

At least two large *anterior lateral cerebral* venous tributaries (Figs. 5 and 6) join each dorso-medial cerebral vein. Smaller and less constant *anterior medial cerebral veins* terminate in it as well. These veins drain the anterior pole of the

Fig. 8. The venous drainage of the medial aspect of the brain of *Testudo geometrica*. (10 × natural size.)

cerebral hemispheres. Laterally its area of drainage is restricted to the regions above the anterior endopyriform sulcus, and, on the medial surface, the region above the septo-cortical sulcus.

The posterior lateral cerebral vein

This large vessel (Figs. 5 and 6) drains the remainder of the dorso-lateral surface of the hemisphere and the upper surface of its posterior pole above the posterior endopyriform sulcus. It joins the dorsal longitudinal vein directly, and more or less opposite the posterior end of the dorsal sac. It may occasionally be joined by the dorso-medial cerebral veins.

The region of drainage of the lateral cerebral veins coincides with that supplied by the middle cerebral artery.

The medial cerebral veins

These (Fig. 8) are relatively constant vessels, which drain the medial surface of the hemisphere above the level of the interventricular foramen. (i) The *anterior medial cerebral group* of small veins join the dorso-medial cerebral veins, before these have united, and drain the anterior third of the hemisphere. (ii) The intermediate third of the medial surface is drained by a larger *intermediate medial cerebral vein* which passes upward and backward and terminates in the vein formed by the union of the dorso-medial cerebral veins or joins the common choroidal vein in those cases where the dorso-medial veins do not unite with one another. (iii) The remaining third of the hemisphere is drained by the still larger *posterior medial cerebral vein*, which runs upward, forward and medially and joins the common choroidal vein just below the anterior pole of the dorsal sac. This vessel also drains the inferior surface of the posterior pole of the hemisphere below the level of the rudimentary amygdaloid sulcus (Fig. 7). It further drains the lower choroidal plexus of the third ventricle which invaginates into the dorsal sac at the lower border of the anterior epiphysial or parapineal body.

The common choroidal vein

On the medial surface of each septal area a vein is formed by the union of a number of smaller tributaries (Fig. 8). This *septal vein* passes upward and slightly backward and thus comes to lie eventually on the upper surface of the supra-commissural recess of the third ventricle. Here it is joined by another vessel, which comes from the regions adjacent to the septo-cortical sulcus. The septal vein then passes directly backward on the dorsal recess. When opposite the interventricular foramen it is joined by the large *choroidal veins*, which emerge from the foramen at the choroidal invagination corresponding to the velum transversum and return blood from the choroid plexus of the corresponding lateral ventricle. The vessel now formed passes upward and slightly backward against the antero-lateral wall of the dorsal sac.

It is joined, at the level of the upper border of the epithalamic eminence, by the corresponding vessel of the opposite side, and thus the *common choroidal vein* is formed.

Just below the anterior pole of the dorsal sac this vessel is joined, on each side, by the posterior medial cerebral vein. Then it proceeds upward and slightly forward to curve round the anterior pole of the dorsal sac. Here it lies in the groove between the two anterior cornua of the sac, so that the latter lie on either side of it. On the upper surface of the dorsal sac it usually joins the vessel formed by the union of the two dorso-medial cerebral veins, if such a vessel is formed. The dorsal longitudinal vein then commences.

The dorsal longitudinal vein

The first part of this large vein (Figs. 5, 6 and 8) lies in the depression of the dorsal surface of the dorsal sac. Here it receives numerous small *tributaries from the upper choroid plexus of the dorsal sac* and also directly from *the*

epiphysial bodies. Beyond the sac the dorsal longitudinal vein continues directly backward over the tectum, cerebellum and fourth ventricle, growing rapidly in thickness as it receives various large venous tributaries in its course over these structures.

Shortly beyond the cerebellum the dorsal longitudinal vein *bifurcates.* The two *terminal branches* then continue posteriorly, laterally and downward over the roof of the fourth ventricle, and leave the endocranial cavity opposite the region of transition from the medulla oblongata to the spinal cord. They terminate in the *internal jugular veins.*

The dorsal longitudinal vein is at no part of its course a sinus comparable to the superior sagittal, or the transverse sinus of the mammalian endocranial vascular system. It has well formed, though thin, walls and is *entirely separate from the dura mater,* deep to which it lies. It traverses the large arachnoid cisterns of the dorsal surface of the brain.

In its course over the dorsal surface of the brain it receives the following tributaries: (i) the small veins from the *dorsal sac, choroid plexuses* and the *epiphysial bodies*; (ii) the small tributaries from the *dorsal surface of the tectum*; (iii) the *anterior mesencephalic and diencephalic vein*; (iv) the *posterior mesencephalic and central cerebellar vein*; (v) the *anastomotic vessel* with its *anterior medullary, cerebellar* and *choroidal branches*; (vi) the *posterior medullary vein*; (viii) the *spinal veins.*

The small veins from the dorsal surface of the dorsal sac, the epiphysial bodies and the tectum need no further description.

The anterior mesencephalic and diencephalic vein

The *diencephalic branch* of this vein (Figs. 5 and 6) drains the whole of the lateral surface of the diencephalon, including the hypothalamic region by means of a special *hypothalamic branch* (Figs. 6 and 7). It is therefore also the larger tributary. The *anterior mesencephalic component* drains the anterior half of the optic lobe (*anterior tectal branch*) on each side, and joins the diencephalic component against the side of the dorsal sac, in front of the lobe. The common vessel then formed reaches the dorsal longitudinal vein behind the posterior pole of the dorsal sac.

The posterior mesencephalic and central cerebellar vein

This vein joins the dorsal longitudinal vein much further posteriorly, i.e. opposite the cerebellum. It is large, and drains a very extensive area of the external surface. Thus the *posterior mesencephalic component,* which is the main branch, is divisible into a *posterior tectal branch,* draining the posterior half of the optic lobe (Figs. 5 and 6), and a *basal mesencephalic branch,* which drains the remaining parts of the midbrain as well as the interpeduncular region (Figs. 6 and 7). The *central cerebellar branches* are small and drain approximately the central third of the anterior surface of the cerebellum

(Fig. 5). Occasionally the posterior mesencephalic and the central cerebellar veins do not unite to form a common vein, and then they reach the dorsal longitudinal vein independently.

The anastomotic vein

This large vein (Figs. 5, 6 and 7), which unites with the dorsal longitudinal vein or its bifurcations opposite the free edge of the cerebellum, is probably identical with that described by Van Gelderen in his numerous studies of the endocranial venous sinuses of vertebrates. In addition to constituting an anastomotic communication between the dorsal longitudinal vein and the internal jugular vein, it also receives several important tributaries from the brain.

A small *lateral cerebellar branch* joins it near its junction with the dorsal longitudinal vein. This branch drains approximately the corresponding lateral third of the cerebellum. It may occasionally remain separate and unite directly with the dorsal longitudinal vein. In the course of the anastomotic vein over the choroid plexus of the fourth ventricle, where it shows a pronounced backward angularity, small *choroid vessels* enter it.

In the angle between the cerebellum and the medulla it is joined by the *anterior medullary vein*, which drains the medulla oblongata as far backwards as the level of the eighth or ninth cranial nerve roots.

The large anastomotic element of the vein may finally be traced forward, downward and outward, until it ultimately leaves the endocranial cavity. During its course it passes between the trigeminal and facial nerves (Fig. 6).

The posterior medullary vein

This is a small vein (Figs. 5 and 6), which drains the remaining portions of the medulla oblongata, terminating in the corresponding terminal branch of the dorsal longitudinal vein as it passes downward and posteriorly opposite the region of transition from medulla oblongata to spinal cord.

The spinal veins

Each bifurcation of the dorsal longitudinal vein receives *dorsal* and *ventral* spinal veins, which enter the endocranial cavity from the spinal canal (Figs. 5, 6, 7 and 8). These veins drain the first part of the cervical spinal cord.

Summary of the venous drainage of the brain

Tributaries of the dorsal longitudinal vein:

(1) The dorso-medial cerebral vein.
  (*a*) Lateral olfactory vein.
  (*b*) Medial olfactory vein.
  (*c*) Anterior lateral cerebral veins.
  (*d*) Anterior medial cerebral veins.

(2) The common choroidal vein.
  (*a*) Septal vein.
  (*b*) Choroidal veins. (Lateral ventricles.)
  (*c*) Intermediate medial cerebral veins.
  (*d*) Posterior medial cerebral veins.
  (*e*) Veins from the choroid plexuses of the dorsal sac.
(3) The posterior lateral cerebral vein.
  (*Note*: May be joined by the dorso-medial cerebral veins.)
(4) The veins from the dorsal sac.
(5) The anterior mesencephalic and diencephalic vein.
  (*a*) (i) Diencephalic branch.
    (ii) Hypothalamic branch.
  (*b*) Mesencephalic branch.
    (i) Anterior basal mesencephalic branch.
    (ii) Anterior tectal branch.
(6) The dorsal tectal veins.
(7) The posterior mesencephalic and central cerebellar vein.
  (*a*) Mesencephalic branch.
    (i) Posterior tectal branch.
    (ii) Basal mesencephalic branch.
    (iii) Interpeduncular branch.
  (*b*) Central cerebellar branch.
(8) The anastomotic vessel.
  (*a*) Lateral cerebellar branch.
  (*b*) Choroidal branches. (Fourth ventricle.)
  (*c*) Anterior medullary branch.
  (*d*) Anastomotic continuation.
(9) The posterior medullary veins.
(10) The spinal veins.
  (*a*) Dorsal.
  (*b*) Ventral.

DISCUSSION

The arterial system

Reptilian Homologies

In the rather scanty literature on the arterial system of *reptiles* we find Hofmann's description (1900) of some of the brain arteries of *Testudo graeca* and of crocodiles; Beddard's discussion (1905) of those of Sauropsida as a whole and of Lacertilia in particular and Dendy's discussion (1909) of those of *Sphenodon*. Quite recently we find some articles on the subject by Kappers (1933) who uses *Chelone midas* as type, and by Abbie (1934), who more particularly discusses *Sphenodon punctatum*.

From the descriptions cited above, Kappers's (1933) in particular, it appears that the *ophthalmic* and *hypothalamic* branches of the carotid are as a rule easily distinguishable. Their significance as fundamental vascular elements is thus well established at the reptilian stage of evolution.

The ***anterior cerebral artery*** in *Sphenodon punctatum* passes forward close to the midline and to the medial side of the olfactory tubercle and disappears by passing on to the medial surface at the coronal sulcus, thus supplying only a very limited portion of the inferior surface of the olfactory bulb. The greater part of the external surface of this structure is apparently supplied by the middle cerebral artery. The anterior cerebral artery is therefore relatively small in *Sphenodon*. In *Chelone midas*, on the contrary, Kappers (1933) maintains that the anterior cerebral is relatively large, and that it supplies the *greater part* of the external surface of the olfactory bulb. From his diagrams, however, it seems that the actual condition is similar to that of *Sphenodon* and that the middle cerebral artery supplies the lateral and the inferior aspects of the olfactory bulb. In *Testudo geometrica* the middle cerebral does *not* contribute to the vascular supply of the olfactory bulb, it being supplied by branches from the anterior cerebral only. Apparently then, as far as has been determined in the reptile, no communication between the anterior cerebral arteries exists. Neither is there any fusion to form a single vessel, as may occur in mammals (Watts, 1934) and as occurs in the case of the posterior cerebral arteries in *Dammonia subtrijuga* (Kappers, 1933).

The course of the anterior cerebral on the medial surface of the hemisphere has not yet been described adequately in any of the reptiles and no detailed comparisons are therefore possible. Abbie, however, mentions that the paraterminal body and the anterior portion of the hippocampus are supplied by it.

In the *Chelone* description (Kappers) terminal branches are depicted on the dorsal and dorso-lateral aspects of the olfactory bulb. But no branches are continued on to the hemisphere as in *Testudo geometrica*.

The evidence of these comparative data, although scanty, supports the conclusion arrived at in this study of the distribution of the anterior cerebral artery, that the artery supplies, roughly, the "palaeo-olfactorium" (Dart, 1925), i.e. the tuberculum olfactorium and palaeostriatum in part, the diagonal band region, the septal formation, (the anterior portion of the hippocampus), the lamina infra-neuroporica and supra-neuroporica, the ventral (and dorsal) commissures, and the lower (and upper) halves of the olfactory bulb. The evidence also suggests that these regions, with the exception of those in parentheses, are usually supplied solely by this artery.

This latter fact is in agreement with the conclusions of Abbie and Shellshear about the "neuro-vascular unity" of nervous structure. But such correspondence between neural and vascular elements is not necessarily exact, as is obvious in the case of the areas of distribution of the middle and the anterior cerebral arteries on the lateral aspect of the olfactory bulb. But such over-

lapping occurs at the earlier stages of development, when the various brain structures are well defined from one another, and when no obvious mechanical disturbing influences come into play to modify the vascular pattern. Since the middle cerebral artery is a secondary vessel which develops in the arterial anastomotic network between the posterior cerebral artery (the homologue of the primitive lateral olfactory artery) and the anterior cerebral artery (or primitive medial olfactory artery), minor variations of the factors controlling the morphogenesis of the vascular pattern may determine that the area of distribution of the middle cerebral vessel be encroached on to a greater or lesser extent by the more primitive anterior cerebral. But such encroachment is never so extensive as to disturb the essential relationships of arterial units to functional regions.

In reptilian brains the ***middle cerebral artery*** can always be found lying opposite the endorhinal sulcus, to the floor of which it is firmly attached by means of the penetrating striate branches. In its effort to adapt itself to the curvatures of the sulcus and the disposition of the cell formations of the lateral wall, it undergoes a great degree of variation of form. In *Sphenodon punctatum* it is evenly and only slightly convex upwards. In *Chelone midas* its course is markedly convex upwards so that it comes to lie on the dorsal lateral surface of the hemisphere. In *Testudo geometrica* it is both tortuous and convex upwards.

Whether the various branches which occur so constantly in *T. geometrica* are to be found also in other reptilian forms cannot yet be determined, as no information is available in the literature.

The anastomotic union between the middle cerebral and the posterior cerebral arteries over the posterior pole of the hemisphere (Kappers, 1933; Abbie, 1934) is not evident in *T. geometrica*.

The histological relationships of the branches of this artery are strikingly constant, and the artery is confined in its region of distribution to the pyriform cell mass complex. Its position with reference to the surface of the brain is thus of some value as an anatomical landmark indicative of the extent of various cell masses. Thus the proximity of the artery to the posterior cerebral artery in the case of *Chelone midas* is due to the slight development of the dorsal wall cortical cell masses. In *Sphenodon* again the artery is placed relatively low down on the lateral surface on account of the great development of the dorsal and dorso-lateral cortices.

The mode of origin of the ***posterior cerebral artery*** from the carotids seems to be variable. While in *Chelone midas* it arises generally from the caudal division of the carotid, either immediately beyond the bifurcation of the carotid or some distance away from it, it arises, according to Abbie (1934), from the anterior division of the carotid, near its bifurcation, in *Sphenodon punctatum*. In *Testudo geometrica* it may arise either from the posterior or from the anterior divisions of the carotid.

These findings necessitate a criticism of the interesting interpretation of the

cerebral arteries given by De Vriese (1904). According to her the anterior cerebral artery is a continuation of the cranial branch of the carotid, and the middle cerebral a collateral vessel, while the caudal division of the carotid terminates as the primitive basilar artery, having the posterior cerebral as its collateral. This she regards as the primitive pattern of blood distribution. But this interpretation does not hold good when the posterior cerebral may arise even in reptiles, and originally did arise, from the cranial division of the carotid.

That the posterior cerebral artery did originally arise from the anterior division of the carotid in lower vertebrates is apparent from Abbie's studies of the arteries of *Rana* or the even more recent accounts of the blood vessels of *Amblystoma*, by Roofe (1935) and Herrick (1935). The amphibian pattern seems to persist in a large number of reptiles. But Abbie exaggerates, when he claims that the posterior cerebral artery of reptiles has as yet no direct relation to the caudal division and that it is not until the mammalian stage of evolution is reached that the posterior cerebral artery acquires its connexion with the caudal division.

Abbie's conclusion concerning the factors involved in the establishment of the vascular pattern is, that the maintenance of blood supply to an area from the nearest source is a hydrostatic law and that the dorso-posterior expansion of the hemispheres of the brain, which occurs with the transition from the reptilian to the mammalian type, is thus responsible for the gradual shifting of the stem of the origin of the posterior cerebral artery to the caudal division of the carotid. But the mode of origin of the posterior cerebral artery is *not* rigidly determined by this factor, as can be inferred from those instances in *Testudo geometrica*, where, notwithstanding the relatively great development of the cerebral hemispheres, and especially the posterior poles, the posterior cerebral artery still arises from the anterior division and then turns *backward* for some distance, in order to reach the medial surface of the hemisphere. Had it arisen directly from the posterior division, through one of the anastomotic channels, which are always present, it could have effected the latter purpose more easily in most of these cases.

It seems that this process of shifting of the stem of origin of the posterior cerebral artery from the cranial to the caudal division of the carotid is more directly related to the gradual establishment of the vertebral system of arteries, by means of which a secondary stream of blood assists in supplying the large brain structures, which make their appearance in subsequent evolution. Since the whole brain had originally to derive its blood supply from the carotid vessels, the blood was so distributed that the cranial divisions of the carotids supplied the cerebral hemispheres, by means of the cerebral vessels, and the caudal divisions supplied the remaining brain structures, the volumes of blood directed respectively into each of these two channels being more or less equal. When the hemispheres developed and expanded, the posterior cerebral artery at first either shifted to the caudal division of the carotid and thus deflected some of the blood supply of the posterior portions of the brain on to the cerebral

hemispheres or else the cranial division of the carotid enlarged at the expense of the caudal. But even these readjustments become inadequate in the absence of secondary sources of blood supply to the brain. It has thus always been recognized that, in the reptile, the cerebral hemispheres are relatively less vascularized than the posterior parts of the brain. (The difficulties experienced in staining the anterior parts of the brain are no doubt in part related to this feature.) Such relatively poor vascularization of the telencephalon is also the case in *Amblystoma* (Roofe, 1935), where the hemispheres are as yet relatively poorly differentiated. With further expansion of the brain, such as occurred with the evolution of the mammals, the source of its blood supply became a serious problem. It is therefore quite reasonable to infer that in some cases these new structures were the first to suffer from the inadequate supply, as it is unlikely that the more vital centres would have had their blood supply interfered with. This difficulty was apparently overcome by the development of a vertebral system of arteries. Their appearance not only allowed the whole of the blood stream of the carotid vessels to be directed to the forebrain, but also assisted in supplying the posterior portions of the hemispheres via the posterior cerebral arteries. It is possible that the absence of a vertebral system of arteries helped to check cerebral evolution, which was possible only in those animals in which such a vertebral system became established.

The course taken by the posterior division of the posterior cerebral artery seems to be subject to considerable variation in the reptilian types. In *Testudo geometrica* the artery remains on the medial aspect along the whole of its extent, anastomosing anteriorly with the anterior cerebral artery. But this is not its invariable disposition in reptiles, for Abbie found it to proceed forward along the dorso-medial border of the hemisphere, as far as the anterior pole. This corresponds to the position of the dorso-medial cerebral veins of *T. geometrica*. In *Chelone midas* (Kappers) and *C. imbricata* (De Lange, 1911) it reaches the dorsal surface of the hemisphere opposite the dorsal sac, and it continues forward from here parallel with, but at some distance from the dorso-medial border, terminating anteriorly over the greater part of the anterior pole. Even more different is the condition in *Dammonia subtrijuga* and in the crocodile (Kappers), where the posterior cerebral arteries are fused to form a median dorsal artery, a condition also found in birds.

Although it has not yet been conclusively demonstrated, the posterior cerebral artery probably has some constant relationship to the hippocampal cell formations. This view is supported by the condition in *Testudo geometrica*, but is contrary to the contention of Herrick (1935) that "the distribution of the arteries does not seem to show any significant relation to the morphological subdivision of the brain". In *T. geometrica* the posterior division of the posterior cerebral artery lies in the middle of the hippocampal cell mass. If the relationship in other reptiles is similar, and it seems to be the case in *Sphenodon punctatum* and the Chelonidae, then the position of the artery on the surface of the hemisphere must be indicative of the relative development of this cell

formation and of that of the formations immediately adjacent to it. If then the vessel lies on the medial surface of the hemisphere, as in *Testudo geometrica*, it shows that the hippocampal cortex is either small, or else has been shifted medially by structures adjacent to it in the dorsal wall of the hemisphere. Conversely, a position on the dorsal surface of the hemisphere, as in *Chelone midas*, *C. imbricata*, etc., means that, in the absence of well-developed dorsal cell formations, the hippocampal cell formation has expanded into the dorsal wall of the hemisphere.

Whether the course of the posterior division of the artery corresponds to the sulcus longitudinalis in the alligator (De Lange) has not yet been determined. Where the artery lies against the medial surface of the brain in *Testudo geometrica* a definite impression is certainly present, i.e. the "endohippocampal sulcus". In all probability this sulcus represents the line along which the hippocampal cortex folds when the "dentate" and "ammon" elements are differentiated. In a closely related species of tortoise, such folding of the medial surface of the hemisphere has actually commenced, being specially marked posteriorly, and producing a condition much like that found in lower mammals. In these cases the posterior cerebral artery is embedded deeply in the fold. Whether the artery has a similar position with reference to the analogous fold seen sometimes in the Varanidae has not yet been determined. And whether this relationship of the posterior cerebral artery is merely of topographical interest is also not yet quite evident. It is possible that its presence may have something to do with the mechanism by means of which the folding of the medial wall of the hemisphere is brought about.

Though generally regarded as being the artery of the hippocampal cell formation, the area of distribution of the posterior cerebral artery seems to be very variable in extent. Abbie describes it as also supplying branches to the anterior part of the mesencephalic tectum, the "pyriform lobe" of the hemisphere, the "amygdala" and the posterior portions of the "paraterminal body", as well as contributing to the formation of the choroid plexus. According to Kappers it supplies most of the dorsal part of the hemisphere, as far forward as the hind border of the "lobus olfactorius". These areas do not correspond to those recorded in *T. geometrica*. The septal cell masses in particular in this species receive none of their arterial supply from this vessel.

The branches to the pyriform formation are significant in that the amygdaloid region seems to be supplied exclusively by the middle cerebral artery in the majority of cases, but occasionally, as described above, a small branch may arise from the posterior cerebral at its commencement and it supplies the habenular radiation, the portions of the "amygdala" below the level of the amygdaloid sulcus, and it terminates on the ventro-medial aspect of the posterior pole of the hemisphere. This corresponds more or less to the area of supply of the inferior cerebral artery. There is, however, no separate inferior cerebral artery, as described by Dendy (1909) for *Sphenodon punctatum*, unless the anterior division of the posterior cerebral artery is equivalent to it.

There are also no signs of the "anastomotic chain" between the cranial division of the carotid and the posterior cerebral artery, such as is found in the crocodile and which is regarded by Abbie as representing the essential reptilian pattern.

In the brain of *Testudo geometrica* the ***diencephalic artery*** very seldom supplies more than the diencephalon. In fact, in this type the diencephalic structures appear to constitute an entirely separate vascular unit of the brain. Whether a diencephalic vessel is recognizable in all other reptilian brains would be important to know. Abbie, in describing such an artery in *Sphenodon punctatum*, states that it also supplies the mesencephalon.

Further, whether ***mesencephalic, cerebellar, choroidal*** and ***medullary vessels,*** such as have been described for *Testudo geometrica*, occur also in other reptiles has not yet been determined. In some of De Lange's photographs (1911, 1913) of the brain of *Chelone imbricata*, medullary vessels, similar to those of *Testudo geometrica*, may be seen.

Vertebrate Homologies

It must be noted that little information is available on the endocranial blood vessels of vertebrates in general, even less than is obtainable on the reptilian vascular system. Kappers (1933) and Abbie (1934) discuss various homologies in vertebrates, and two good accounts of the endocranial blood vessels of *Amblystoma* are given by Roofe (1935) and Herrick (1935).

The constancy of occurrence of the ophthalmic artery has been demonstrated by Kappers. And Roofe has found that this artery may arise extracranially in *Amblystoma*. The hypothalamic artery is also constantly present, but its mode of origin is variable. The bifurcation of the carotids into cranial and caudal divisions appears to be a constant feature in all vertebrates.

There exists some doubt as to whether an anastomosis between vertebral arteries and the caudal divisions of the carotids is established during the early stages of evolution. Fusion of these caudal divisions of the carotids into a single median artery is not favoured at the earliest stages of evolution, and the condition of these vessels in the brain of *Testudo geometrica* is primitive. Thus Roofe found that in *Amblystoma*, in a fair percentage of cases, the "basilar" is represented by two vessels, with anastomotic bridges between them. Abbie described the "basilars" as separate paired vessels in *Rana*. De Vriese (1904) is definitely in favour of the view that in the early evolutionary stages there are two basilar arteries. Moreover, she finds that this is the case in the early stages of mammalian embryonic development. Transverse anastomosing vessels subsequently develop and still later loops are found along the course of the two basilar arteries. Finally, through further development in some places and atrophy in others, a single median basilar artery results.

This process of the formation of the single basilar vessel is related to the development of the vertebral system of arteries. When the dominance of the

latter arteries over the posterior portions of the brain is established, all that remain of the caudal divisions of the carotids are the small posterior communicating arteries. Roofe found a "posterior communicating ramus", which is constantly present so as to close the "circle of Willis" posteriorly. But no communication between the anterior cerebral arteries was found.

In the earlier stages of evolution each cranial division of the carotids may apparently be either a large trunk, which, after a short course, subdivides into the cerebral branches (Abbie) or the cerebral arteries may arise independently from the carotid (Kappers). Their area of distribution is restricted at first and some degree of economy is observed in the method of blood distribution in that they are symmetrically placed on the forebrain and very well interconnected by means of anastomotic channels, thus forming a closed vascular network. This anastomotic plexus, according to Abbie, is the primitive arterial pattern of vertebrates.

Without doubt the "medial olfactory artery" of the fish brain evolves into the anterior cerebral artery of the reptiles. This has been very well demonstrated by Kappers, who prefers calling the vessel the anterior cerebral artery throughout. It supplies the "palaeo-olfactorium" of Dart (1925) in all these types and only at more advanced stages of evolution does it contribute to the supply of adjacent regions.

About the homologies of the other two cerebral arteries there is less certainty. In the fish and amphibian forms Abbie describes no middle cerebral but only a "lateral olfactory artery". This corresponds to the "lateral-olfacto-bulbar" artery of the plagiostomes, which Kappers also describes as the middle cerebral artery in both fishes and amphibians, and which he considers to be homologous with the middle cerebral artery of reptiles and with the "pyriform artery" of mammals. Abbie, on the contrary, homologizes the lateral olfactory artery of fishes with the "striatal branch of the lateral olfactory artery" of amphibians, and the striatal branch of the middle cerebral artery in reptiles. According to him the middle cerebral artery appears as an entity for the first time in reptiles. He postulates an ancestral form, which is intermediate between that of the fishes and the reptiles, and which has since disappeared. In the amphibians the middle cerebral is represented by the "striatal and pyriform branches of the lateral olfactory artery". This view seems acceptable, especially if not only mere morphological resemblance, but also constancy of relationships of brain arteries be regarded as essential criteria to be taken into consideration in coming to a decision regarding homologies.

Roofe does not find an arrangement of the branches of the cranial division of the carotid similar to that described by Abbie. But it is difficult to homologize the individual elements of the two descriptions. Roofe's "ramus hemisphaerii ventralis" is evidently equivalent to Abbie's "lateral olfactory artery" plus his "medial olfactory artery". The "ventro-medial branches" to the septal, pre-optic and bulbar regions and to the "anterior olfactory nucleus" in *Amblystoma* may possibly be represented by the "medial olfactory artery" of

Abbie, but the evidence is insufficient to justify a definite statement. It seems possible, however, that the most anterior of the "ventro-lateral branches" of the "ramus hemisphaerii ventralis" in *Amblystoma* may, in view of their distribution to the anterior portion of the "nucleus olfactorius dorso-lateralis", the "striatal" region, the lateral surface of the "anterior olfactory nucleus" and the olfactory bulb, be equivalent to and therefore homologous with the middle cerebral artery of reptiles. It would then correspond to the whole of the lateral olfactory artery of Abbie, including both pyriform and striate branches. In *Rana* there is no vessel similar to the large, more posterior, ventro-lateral branch of the "ramus hemisphaerii ventralis" described by Roofe in *Amblystoma* and distributed to the "nucleus olfactorius dorso-lateralis" the "striatal" region and the "amygdala". This is possibly related to the poorly developed "amygdala" in *Rana*. In reptiles, e.g. *Testudo geometrica*, it is undoubtedly represented in part by the vessels of supply to the regions adjacent to the rudimentary amygdaloid sulcus and to the large branch of the middle cerebral artery, which is found in the posterior endo-pyriform sulcus.

The rudiments of the posterior cerebral artery appear, according to Kappers, as an independent element at an early evolutionary stage, e.g. in the selachian *Laemargus*, where it is distributed over the "hippocampal primordium". In *Rhina* it is better developed and has nearly attained the form and character of that of the reptilian. Abbie, however, avers that in the amphibian several "hippocampal arteries" appear as branches of the "lateral olfactory artery" and at subsequent stages these acquire a nearer source of origin through a more posterior anastomosis with the cranial division of the internal carotid, so that a single hippocampal or posterior cerebral artery then arises from the latter.

The "ramus mesencephali superior" of *Amblystoma* is plainly homologous with the *mesencephalic artery* of *Testudo geometrica*. Their mode of origin and manner of distribution are very similar. This is no doubt due to the fact that the structures supplied in each case are morphologically similar. The branch to the epiphysial bodies in the dorsal sac of the third ventricle is, however, not described in *Amblystoma*. The dorsal surface of the cerebellum, as in the case of *Testudo geometrica*, also derives its supply from the "ramus mesencephali superior". Herrick (1935) in addition describes in *Amblystoma* an "arteria choroidea rhombencephali superior", which arises from the "ramus mesencephali superior". Roofe also describes an "arteria choroidea ventriculi quarti", which appears to arise from the caudal division of the carotid, in the vicinity of the ninth cranial nerve.

Roofe's artery to nerves V and VI arises in *Testudo geometrica* as a distinct branch of the *cerebellar artery*. The latter again, as in *T. geometrica*, arises in *Amblystoma* as a distinct vessel, which passes upward and backward anteriorly to the oculomotor nerve in Fig. 4 (Roofe) and Fig. 3 (Herrick), but posteriorly to it, as in *Testudo geometrica*, in Fig. 2 (Herrick).

The "arteria auditiva" of *Amblystoma* is represented in *Testudo geometrica* by branches from the stem of the artery to the choroid plexus of the fourth ventricle.

The more caudal vessels, of which two chiefly are stressed by Roofe, i.e. those opposite nerves IX and X are evidently arranged in a "segmental fashion". The *first spinal arteries* are obviously comparable in *Amblystoma* and *Testudo geometrica*. They mark the caudal end of the medulla oblongata and give rise to the lateral spinal arteries.

General Features of the Arterial System

As must be evident from the above descriptions, the pattern of the arterial system of *T. geometrica* is that of a segmental or pseudosegmental distribution of the vessels. The vascular segments along the medulla oblongata demarcated by the branches of the caudal divisions of the carotid, in particular, are so evenly spaced and so like the morphological "segments" of the spinal cord, that a metameric type of segmentation is suggested. Whether this is actually the pattern for reptiles in general is, however, still unknown. It is also obscure whether these segments or pseudo-segments are actually of a metameric nature or origin, or whether their arrangement corresponds to the neural metamerism discussed by Kingsbury (1922).

The morphological "segments" into which the spinal cord is apparently divided correspond to the mesodermal metameres and may be regarded as resulting in a mechanical way from their presence and direct relationship. They are probably not true segments, in the sense, for instance, that the mesodermal tissues of the vertebrate body are segmented. The relationship of the cranial mesodermal somites to the cranial nerves is more indirect and the mechanical influences on the form of the brain therefore less evident. Moreover, these parts of the brain have also specialized to a very great degree, so that the segmental character of the brain, which is seen at the earliest stages of evolution, has now been lost. And yet, the "segmental" or "pseudo-segmental" arrangement of the blood vessels persists to a certain extent even in the reptile. This may perhaps be explained on the supposition that these brain vessels have their segmental character determined at an early stage of development, while the embryo is still very definitely segmented, and before any great change has occurred in the brain substance itself. At later stages of embryological development, when the mesodermal somites, particularly the myotomes, have migrated to such a degree that their segmental arrangement has disappeared and their mechanical influence on the form and structure of the neural tube is greatly reduced, the arteries still retain a slightly modified segmental arrangement. The evidence of segmentation in the neural tissues, however, is lost with their expansion and ontogenetic development.

The mechanical influences on the pattern of blood distribution, as contrasted with the neuro-vascular unity concept of Shellshear, are emphasized.

Undoubtedly, many vascular arrangements, which are difficult to explain in terms of hereditary influences or which are based on the constancy of functional neuro-vascular relationships, are probably produced in accordance with the as yet not fully understood laws of hydrostatics, as far as they concern the blood vascular system. Whether the hydrostatic features of the problem should be considered as secondary to the influences of segmentation or of hereditary functional patterns, remains uncertain. Certainly, on examining the vascular pattern of the brains of various animal types, it is evident that it is as likely that the distribution of the fore-brain arteries especially is purely hydrostatically determined, as that hereditary and functional neuro-vascular relationships should have a decisive influence on the morphogenesis of the vascular pattern. These patterns at various evolutionary stages undergo considerable mutation. At the reptilian stage (Herrick, 1935) the ground-plan of an apparently successful pattern, which persists with only slight subsequent modification, is evolved. This suggests that some purely physical forces are to a large extent responsible.

Apart from the segmental manner of distribution of brain vessels, which may also be explained on a mechanical basis, it is clearly evident in some instances in this brain that some hereditary influence, which aims at retaining early established neuro-vascular unity throughout evolutionary development, is still active in the manner suggested by Shellshear. It is enough to mention, in illustration, the manner in which the epiphysial bodies, which are embedded in the roof of the dorsal sac and which can only be mesencephalic derivatives, are supplied by the mesencephalic artery. The epiphysial branch of this vessel, as shown above, originates dorsally opposite the interval between the tectum and the cerebellum, and courses forward from here, below the dorsal longitudinal vein, eventually to reach the dorsal surfaces of the epiphysial bodies in the dorsal sac. The rest of the dorsal sac is supplied by the diencephalic artery.

There is the further possibility, in connexion with the problem of the mechanism of the establishment of the vascular pattern, that the need for greater vascularity in any region may decrease the resistance to blood flow in that direction, through the opening up of anastomotic plexuses and the increase in and growth of capillaries. Such increased vascularization arises as the result of greater functional activity and attendant developmental changes in a part, and must obviously keep pace with such expansion in the part. Under these circumstances the supply will come from the vessels already supplying the part. Thus the derivatives of a primordial structure will have an identical source of supply, and this constancy of vascular supply of neural derivatives from a uniform source forms the basis of Shellshear's neuro-vascular unity concept. Roofe, having found the individual sources of vascularization in *Amblystoma* variable, holds a different view.

The existence of regions of relatively greater or lesser functional activity in the brain is important. That any actively functioning region in the animal body

very soon acquires a high vascularization has been conclusively proved from studies in embryology, physiology and pathology. But how this vascularization is established has not yet been satisfactorily determined. Two distinct possibilities exist. On the one hand, an accidentally relatively greater vascularity of any one portion of the brain may have resulted in an expansion and development of this portion, or, on the other hand, the development and growth of such a region necessitating a more liberal blood supply caused an expansion of the blood vascular system of that region. The first alternative is certainly opposed to many findings with regard to the part played by the relative blood supply in morphogenesis. But Roofe and Herrick do suggest such a possibility. If the second alternative were the dominating condition, it is obvious that the rate of vascularization had to be proportional to the rate of development of the neural tissue. It is interesting then to consider, in this connexion, what effect an inadequate vascular supply must in the past have had on the development of the brain, were such a contingency to have arisen. The establishment of the vertebral arterial supply to assist the carotids in supplying the expanding brain structures, certainly prevented this from occurring in the case of the forebrain during the stages of transition from the reptilian to the mammalian types.

The venous system

The available sources of information regarding the venous systems of vertebrates in general are threefold. First, there are the excellent accounts of the venous drainage of *Amblystoma*, given in the papers by Roofe (1935) and Herrick (1935), which have already been extensively referred to in connexion with the arterial system. Next there is the comparative anatomical information regarding the endocranial venous sinuses, which is widely discussed by Van Gelderen (1923–7) as well as by O'Donoghue (1910, 1912, 1914, 1920), Dendy (1909) and Goodrich (1932). Finally there are the detailed textbook descriptions of the various systems in man and some of the higher mammals.

(*a*) Herrick and Roofe supply detailed accounts in the case of the venous system as well. Although the arterial elements of the blood vascular system of the *Amblystoma* and *Testudo geometrica* brains show some degree of correspondence, as has been demonstrated, there is even greater similarity between the venous drainage systems in these two types. The following homologies are permissible.

The lateral olfactory vein agrees closely with the anterior part of the "vena hemisphaerii dorsalis" of *Amblystoma*. Both drain the dorso-lateral surfaces of the olfactory bulbs and the "anterior olfactory nucleus" (or its equivalent in *Testudo geometrica*). But in *T. geometrica* this vein drains also a large part of the striatal region and a more extensive area of the lateral surface of the bulb.

The dorso-medial cerebral vein plainly resembles the posterior continuation of the vena hemisphaerii dorsalis in *Amblystoma*. It receives tributaries from

the medial aspect of the olfactory bulb and hemisphere and from the anterior end of the dorso-lateral surface in an almost identical manner to what happens in the case of the ramus hemisphaerii dorsalis. While the latter terminates in the nodus vasculosis, the dorso-medial cerebral vein of *Testudo* joins its fellow of the opposite side and this single vessel unites with the common choroidal vein to form the dorsal longitudinal vein.

The "venae hemisphaerii ventro-mediales" of *Amblystoma*, though variable, correspond to the veins draining the medial surface of the hemisphere in *Testudo*. Roofe describes two groups of vessels. The dorsal group drains the medial side of the olfactory bulb, the "anterior olfactory nucleus" and the "primordium hippocampi" in *Amblystoma*. The vessels equivalent to these in *Testudo* are the medial olfactory vein, the anterior medial and the intermediate medial cerebral veins. The ventral group of the venae hemisphaerii ventro-mediales of *Amblystoma* drain the "septal, pre-optic and the strio-amygdaloid" areas, and more anteriorly also the ventral surfaces of the olfactory bulb, and the "anterior olfactory nucleus". They may be homologized in part with the septal vein and the medial olfactory vein.

The "venae taeniae", described by Roofe, seem to correspond to the posterior medial cerebral vein.

The dorsal longitudinal vein of *Testudo* is equivalent to the "nodus vasculosis", the "oblique sinuses" and the "rete sacci endolymphatici" of *Amblystoma*, for these serve the same purpose.

The posterior lateral cerebral vein drains areas homologous with those drained by the "vena hemisphaerii posterior" of *Amblystoma*.

The diencephalic and anterior mesencephalic tributary of the dorsal longitudinal vein of *Testudo geometrica* corresponds to the "vena prosencephali lateralis". But it also receives the hypothalamic vein, which ends in *Amblystoma* in the "rete sacci endolymphatici" instead.

The posterior mesencephalic and central cerebellar vein is apparently equivalent to the "vena mesencephali" and the "vena basilaris anterior" in *Amblystoma*.

(*b*) Blood vessels homologous with only the larger veins of the *Testudo geometrica* pattern have been discussed in the works of Van Gelderen, O'Donoghue, Dendy, Goodrich and others.

The veins in *T. geometrica*, as pointed out above, are not sinusoidal in character. In this they differ from some which have been described by Van Gelderen. This author (1924) describes a secondary dura mater in *Lacerta*, which is formed by the gradual fusion of the true dura mater and the endostium of the cranial bones. The veins, which in earlier ontogenetic development were to be found between these layers, are thus transformed into peridural venous sinuses. In *Testudo geometrica* the endocranial veins, which have been described here, were only displayed properly after complete removal of the dural coverings. That they are true veins and lie in the interarachnoidal or the arachnoidal spaces was confirmed on histological examination. They are therefore strictly

comparable with the intermeningeal veins of fishes. Peridural vessels can be displayed. In the endocranial cavity these are minute. In the vertebral canal, however, complicated plexuses are found embedded in the peridural tissues, and these must be carefully distinguished from such perineural veins as for instance the spinal tributaries to the bifurcations of the dorsal longitudinal vein in *T. geometrica*.

The pattern of the larger venous elements in *T. geometrica* differs to a certain extent from the patterns which are available for comparison.

The dorsal longitudinal vein is undoubtedly homologous with the "sinus longitudinalis prosencephali-mesencephali-rhombencephali" of reptiles. (In his text Van Gelderen frequently refers to this vessel as a vein.) This longitudinal sinus must also include part of what has been called the dorso-medial cerebral veins in *T. geometrica*. In various illustrations it is seen to extend far forward on the dorsal surface of the brain. In the case of *Tropidonotus natrix*, as figured by O'Donoghue, it extends even further forward than the coronal sulcus. If a distinction between the dorso-medial cerebral veins and the dorsal longitudinal vein exists, it would be better to indicate this, as the two vessels undoubtedly have totally different morphological significance.

The dorsal longitudinal vein terminates posteriorly by bifurcating and the branch thus formed on either side is homologous with the corresponding "vena occipitalis" of Van Gelderen. No branch equivalent to the "vena cerebralis posterior" was found in this brain. Van Gelderen and Goodrich, however, state that both a vena occipitalis and a vena cerebralis posterior persist in Chelonia.

Van Gelderen homologizes the longitudinal sinus of reptiles with the closely similar structures in higher vertebrate types, e.g. the superior sagittal sinus in man. Thus he states (1923) that, depending almost entirely on the morphology, the original "sinus longitudinalis prosencephali-mesencephali-rhombencephali" of tetrapods becomes gradually restricted so that only a "sinus prosencephali" (the superior sagittal sinus) remains in the mammal. Again in 1925 he describes a human abnormality (Fig. E), where the "sinus occipitalis" (which latter is a progressive feature in so far as it occurs only in the human brain) enlarges at the expense of the "sinus transversus", which has disappeared. He states emphatically that this abnormal "vena longitudinalis prosencephali-rhombencephali" is strictly comparable with the similar sinus in Sauropsida. It is doubtful, as will be seen presently, whether these latter homologies are correct.

Van Gelderen states that the anastomotic vein is constantly present in higher vertebrates; he has discussed its comparative features fully. In *Testudo geometrica* the vein descends behind the trigeminal nerve. In differing thus from *Chrysemys marginata* and other Chelonia, where the vein passes in front of the trigeminal nerve, the terminal portion of the vein must correspond to the "vena cerebralis medialis" of Van Gelderen. In *Testudo geometrica* the vein is also relatively small, for most of the venous blood, as in the case of the crocodile, appears to leave the skull posteriorly via the dorsal longitudinal vein.

The lesser veins described in *T. geometrica* and which have been compared with those of *Amblystoma*, have apparently not yet been described in other reptiles.

(*c*) On comparing the venous pattern of *Testudo geometrica* with that of the human brain, it is immediately evident that hardly any of the mammalian superficial cerebral vessels have appeared at the reptilian stage of evolution. The reptilian endocranial veins represent only the deeper vessels of the human brain. Taking this into consideration it is a relatively simple matter to homologize the various elements of the respective vascular patterns. This is the easier as the pattern of the deeper veins of the human brain is very constant on account of the relative constancy of the morphology of the neural structures which they drain.

The deep veins of the human brain, which are of special interest, are the choroidal vein, the terminal vein, the internal cerebral vein, the great cerebral vein, the septal veins and the inferior striatal veins. A homologue for each of these may be found amongst the veins described in *T. geometrica*. This is not surprising when one considers that the regions of the mammalian brain, which these vessels drain, are already present in the reptile, even though as yet only in a rudimentary form.

The choroid veins of *T. geometrica* undoubtedly represent the internal cerebral veins. They drain the choroid plexus of the lateral ventricles and also its walls. It is actually therefore constituted from tributaries which in the adult venous pattern are represented by the terminal vein. It drains the thalamus and the caudate nucleus as well as receiving the choroid vein, which emerges along the medial edge of the choroid plexus which is drained by it. These tributaries in the reptilian brain do not, however, form such distinct vessels as the terminal veins or the choroidal vessels, although the rudiments of these vessels can be recognized. With regard to the rudimentary character of the reptilian representative of the terminal vein, it is apparent that the thalamus and the striatal cell masses have not yet developed fully enough in reptiles to form a continuous groove between them, in which a large, single, forwardly coursing vessel could lie. The venous drainage of the choroid plexus is even more distinctly developed and exists as a system of veins, which join with the terminal vein rudiment to form the choroidal vein, which then issues from the interventricular foramen.

It is difficult to decide on the homologue of the septal vein. While the terminal vein of the lateral ventricle is described as receiving a septal branch before joining the choroid vein to form the internal cerebral vein, it is clear that this septal branch drains the internal surface of the septum and is thus not the same vessel as the septal vein of the reptile. In *T. geometrica*, on the other hand, no single vein draining the ventricular surface of the septal region can be distinguished. Only occasional minute venous tributaries are found. If the septal branch of the terminal vein of the human brain cannot be regarded as homologous with the septal vein of *T. geometrica*, it is quite inconceivable, in

view of the relative constancy of the vascular pattern, that the latter should disappear without leaving any indication as to its fate, unless a sufficiently gross disturbance of the architectural arrangement of the brain occurred in the transition from the reptilian to the mammalian brain types, to account for its disappearance.

The formation of the corpus callosum and the septum pellucidum must be chiefly responsible for the disappearance of the reptilian septal vein. It is obvious, that with the backward growth of the corpus callosum over the roof of the third ventricle any branches of the internal cerebral veins draining at first into them, from the septal region for instance, begin to experience difficulty in reaching these veins. The drainage of these regions is therefore necessarily into more accessible channels. This is possible on account of the existence of the basal veins in the human brain, into which most of the blood from these regions is directed. It would be interesting to know the arrangement of the blood vessels in this region in acallosal brains, where no such disturbances have obviously occurred.

The common choroidal vein of *T. geometrica* resembles in all respects the great cerebral vein of the human brain. It receives blood from the internal cerebral veins or their equivalents and ends in the dorsal longitudinal vein in the reptile in the same way as the great cerebral vein ends in the straight sinus in the human brain. In both cases the vessel is very well developed. There are, however, no definite basal veins yet which form such important tributaries to the great cerebral vein in the adult human brain. But, as is the case for the terminal vein, the ground plan for these is already established in the reptile in the form of either the posterior medial cerebral veins or the diencephalic veins with their hypothalamic branches or even the dorso-medial cerebral veins.

As has already been pointed out, Van Gelderen, O'Donoghue and others have not distinguished very clearly between the dorsal longitudinal vein and the dorso-medial cerebral veins as separate elements of their "sinus longitudinalis prosencephali-mesencephali-rhombencephali". The prosencephalic element (dorso-medial cerebral veins) has further also been homologized with the superior sagittal sinus of the mammalian brain (Van Gelderen, 1924). This latter homology, however, is not acceptable. It must be recognized that the brain regions, which are drained by the dorso-medial cerebral veins, are displaced posteriorly, medially and inferiorly with the expansion of the neopallium and the backward growth of the corpus callosum. It is not unlikely that these veins were similarly displaced, and if this happened, it is possible that they are homologous with the basal cerebral veins. The fact that the olfactory tributaries of the dorso-medial cerebral veins of the reptile drain into these basal cerebral veins in mammals supports this view.

The inferior and the superior sagittal sinuses develop in the embryonic mammalian from longitudinal anastomoses of the anterior and the middle venous plexuses, which have no primary connexion with the great cerebral veins or the straight sinus.

The mesencephalic and rhombencephalic part of the sinus longitudinalis, which is equivalent to the dorsal longitudinal vein of *T. geometrica*, is stated by Van Gelderen to disappear in higher mammals. But he does not discuss its relationship to the great cerebral vein and the straight sinus of mammals, with which it has many features in common. The whole of it is perhaps not strictly homologous with the straight sinus, for in running backward over the tectum and the cerebellum it also receives tributaries from them. The terminal part of the great cerebral vein is probably also derived from it originally. It is not a sinus, but a definite vein. It receives the common choroidal and the dorsomedial cerebral veins, which have been compared with the great cerebral vein and the basal veins respectively. The next step in the evolution of the straight sinus from this dorsal longitudinal vein is obviously the formation of the inferior sagittal sinus and the establishment of a connexion posteriorly with the dorsal longitudinal vein.

The large vessels draining into the dorsal longitudinal vein cannot directly be compared with the veins draining the midbrain and cerebellum of the mammalian brain. The mammalian tectal veins are relatively insignificant and terminate for the most part either in the great cerebral vein or in the basal veins. The mammalian cerebellar veins, which have been described, are larger, more numerous, and cannot be homologized with those of *T. geometrica*, as they are restricted to the hemispheres of the cerebellum. The reptilian cerebellar veins represent the veins draining the older central parts of the cerebellum. The fate of the anastomotic vein has been fully discussed by Van Gelderen.

(*d*) With regard to the problem of neuro-vascular unity it is of interest to note that the brain can to a certain extent be mapped out into territories of relatively constant distribution, from which the venous return is into a similarly constant pattern of veins. These regions are not necessarily the same as those which the arteries supply, there being considerable overlapping. This lack of correspondence suggests that totally different conditions and influences may be responsible for the establishment of the venous and the arterial patterns respectively. This is emphasized by the fact that in spite of the seeming constancy of the venous pattern in the same species, remarkable differences may be noted in brains of different vertebrate groups where the actual neural tissues have undergone external modification. This apparent correlation between the venous pattern and the form of nervous system suggests that mechanical influences are the most important of the factors responsible for the features of the nervous pattern.

There is further a tendency for the veins to coalesce, so that fewer but larger channels are found in higher vertebrates.

SUMMARY

A noteworthy feature of the arterial vascular pattern of this brain is the absence of a vertebral system of arteries, so that all the blood supply is derived from the carotid arteries. The establishment of the vertebral arteries, which

only occurs later, is a necessary step in the evolution of the brain, particularly the forebrain.

The cranial divisions of the carotids most frequently give rise to all three cerebral vessels. Thus they supply the telencephalon only. The caudal divisions supply the remainder of the brain as no vertebral arteries are present.

In describing the course and the distribution of the anterior cerebral artery, it is seen to supply the palaeo-olfactory sensorium, as defined by Dart. Moreover, the olfactory bulbs are supplied by this artery only. There is no communication between these arteries in reptiles, nor any fusion.

The course of the middle cerebral artery depends largely on the distribution of the cell masses of the lateral wall. This artery supplies the pyriform cell complex only and its branches appear to have a constant relationship to the distribution of the cell masses.

Abbie has named the posterior cerebral artery of the reptile and lower forms the hippocampal artery. This is entirely justified, for it is distributed to the whole of the hippocampal cell formation. In this connexion the branches to the amygdaloid region are particularly significant. The mode of origin of the vessel is variable. Originally it arose from the cranial division of the carotid. In the primitive forms it is therefore not a collateral of the caudal division of the carotid, as is claimed for it by De Vriese. But it may already arise from the caudal division in reptiles (cf. Abbie). The shifting of the stem of origin of this artery from the cranial to the caudal divisions of the carotid is probably more directly related to the evolution of the vertebral arteries, in whose presence alone it is a successful change, than to the mechanical disturbances created by the dorso-posterior expansion of the hemispheres, which attends the evolution of the neopallium. The position of the artery, whether on the medial or on the dorsal aspects of the hemispheres is an index of the degree of development of the cell masses of the hemisphere. The relationship of the artery to the endo-hippocampal sulcus, the probable site of later folding of the medial wall of the hemisphere, is an interesting feature. The significance of the anterior division of the posterior cerebral artery is obscure. It is probably homologous with the inferior cerebral artery.

The large caudal divisions of the carotids supply the remainder of the brain by means of the following branches. The diencephalic arteries supply the diencephalon, the dorsal sac and the choroid plexuses of the third ventricle. The mesencephalic arteries supply the optic tectum, the anterior and dorsal surfaces of the intermediate third of the cerebellum and the epiphysial bodies. The cerebellar arteries are small and supply the outer thirds of the plate-like cerebellum. There are branches of supply to the choroid plexuses of the fourth ventricle and the regions of the medulla oblongata corresponding to the cranial nerve roots. The first and the lateral spinal arteries and the interpeduncular arteries also arise from it. Anastomotic bridges between the caudal divisions occur in reptiles, and fusion is a late feature. To some extent it is related to the formation of the vertebral arterial system.

Vertebrate homologies are discussed, but no detailed comparisons are possible, because existing descriptions are still very inadequate.

The relationship of the pseudo-segmental character of the vascular pattern to neural metamerism is discussed. That the primitive segmental pattern should persist to some extent, in spite of the gross transformations which the cranial structures have undergone, can be explained on the basis of the early ontogenetic and phylogenetic development of the pattern.

The influences, which tend to maintain neuro-vascular unity, as expounded by Shellshear, are contrasted with such physical hydrostatic forces as are also concerned in the morphogenesis of the vascular pattern. There is also some correspondence apparently between the degree of functional activity and the intensity of vascularization of a part. The possible effect of defective vascularization of a part in retarding its evolution is discussed.

While the arterial supply of the brain is derived from the ventral aspect of the brain the venous drainage is towards the dorsal surface. The essential venous pattern consists of a dorsal longitudinal vein, which receives separate branches from the olfactory bulbs, the cerebral hemispheres, the choroid plexuses of the lateral ventricle, the diencephalon, the dorsal sac, the mesencephalon, the cerebellum, the choroid plexuses of the fourth ventricle, the medulla oblongata and the cervical spinal cord.

The venous pattern is compared with that of *Amblystoma*, and many homologies could be established. The most important difference is that the well-defined dorsal longitudinal vein is represented in this type by a "nodus vasculosis", which is again related to the "rete sacci endolymphatici".

The reptilian homologies of the larger veins only could be determined. It should be noted that in *Testudo geometrica* these vessels are proper veins and not sinuses. The peridural sinuses are diminutive.

The "sinus longitudinalis prosencephali-mesencephali-rhombencephali" of vertebrates, which is discussed by Van Gelderen, is equivalent to the dorsal longitudinal vein together with the dorso-medial cerebral vein of *T. geometrica*. Although Van Gelderen maintains that in further evolution the mesencephalic-rhombencephalic part of the dorsal sinus, of his description, becomes reduced, and that the prosencephalic element enlarges to become the superior sagittal sinus, it is probable that the reverse occurs. The prosencephalic parts, which represent the dorso-medial cerebral veins, remain relatively small and are pushed posteriorly medially and inferiorly with the rhinencephalon during the expansion of the neopallium. The mesencephalic and rhombencephalic part of the sinus which corresponds to the dorsal longitudinal vein, enlarges and becomes the straight sinus. The inferior sagittal sinus, which connects with the straight sinus, develops later.

There is no "vena cerebralis posterior". The terminal part of the anastomotic vein is equivalent to the "vena cerebralis medialis". As in crocodiles the anastomotic vein is small and the main venous drainage into the internal jugular vein is via the dorsal longitudinal vein.

Probably no representatives of the mammalian superficial cerebral veins have developed, and homologies for the deeper veins only can be found. The choroid veins become the internal cerebral veins and the common choroidal vein forms the great cerebral vein. No definite terminal veins are present. The disappearance of the septal veins is intelligible in view of the profound mechanical disturbances brought about by the development of the corpus callosum. The dorso-medial cerebral veins are probably homologous with the basal veins.

The lack of correspondence between the regions of arterial supply and the areas of venous drainage, the variability of the venous pattern, which occurs with changes in the shape of the brain, and also the constancy of the pattern in the same species, indicate that mechanical influences are probably the most important factors in the morphogenesis of the venous pattern.

EXPLANATION OF ABBREVIATIONS

A.C.A. (*a*) to *A.C.A.* (*m*)	Branches of the anterior cerebral artery.
A.C.A. Ram. Lat.	Lateral division of the anterior cerebral artery.
A.C.A. Ram. Med.	Medial division of anterior cerebral artery.
A.C.A. Inosc. A.C.P.	Inosculation of the anterior and posterior cerebral arteries.
A.C.I. Div. Caud.	Caudal division of internal carotid artery.
A.C.I. Div. Cran.	Cranial division of internal carotid artery.
A.C.M. (*a*) to *A.C.M.* (*i*)	Branches of the middle cerebral artery.
A.C.P. (*a*) to *A.C.P.* (*d*)	Branches of the posterior cerebral artery.
A.C.P. (*a*) *Alt.*	Alternative mode of supply of amygdaloid region.
A.C.P. Ram. Ant.	Anterior division of posterior cerebral artery.
A.C.P. Ram. Post.	Posterior division of anterior cerebral artery.
A.D. Ram. Thal.	Thalamic branch of diencephalic artery.
A.D. Ram. S.D.	Dorsal sac and choroidal branch of diencephalic artery.
A.M. (*a*) to *A.M.* (*c*)	Branches of the mesencephalic artery.
A.M. Ram. Lat.	Lateral terminal division of mesencephalic artery.
A.M. Ram. Med.	Medial terminal division of mesencephalic artery.
A.M.R.L. Cer.	Cerebellar branches of lateral terminal division of mesencephalic artery.
A.M.R.L. Ch.	Choroidal branches of lateral terminal division of mesencephalic artery.
A.M.R.M. (*a*) to *A.M.R.M.* (*c*)	Branches of the medial terminal division of the mesencephalic artery.
An. Br.	Anastomotic bridge between the caudal divisions of the carotids.
Art. Car. Int.	Internal carotid artery.
Art. Cer. Ant.	Anterior cerebral artery.
Art. Cer. Med.	Middle cerebral artery.
Art. Cer. Post.	Posterior cerebral artery.
Art. Cer. Post. Alt.	Alternative mode of origin of posterior cerebral artery.
Art. Cereb.	Cerebellar artery.
Art. Dienceph.	Diencephalic artery.
Art. Epiph.	Epiphysial artery.
Art. Hypothal.	Hypothalamic artery.
Art. Int.-Ped.	Interpeduncular artery.
Art. Mesenceph.	Mesencephalic artery.
Art. Med. Obl.	Pseudo-segmental arteries to the medulla oblongata.
Art. Ophthal.	Ophthalmic artery.
Art. Pl. Ch. Vent. IV	Artery to choroid plexus of fourth ventricle.

Art. Sp. Lat.	Lateral spinal artery.
Art. Sp. Prim.	First spinal artery.
Art. Sp. Vent.	Ventral spinal artery.
Bulb. Olf.	Olfactory bulb.
Cereb.	Cerebellum.
Ch. Opt.	Optic chiasma.
Comm. H.C.	Dorsal and ventral commissures of cerebral hemispheres.
Dienceph.	Diencephalon.
Em. Epithal.	Epithalamic eminence.
Em. Hypothal.	Hypothalamic eminence.
Em. Thal.	Thalamic eminence.
For. Int.-Vent.	Interventricular foramen.
Hem. Cer.	Cerebral hemisphere.
Hypoph.	Hypophysis.
Hypothal.	Hypothalamus.
Inv. B.O. D.-L.	Dorso-lateral bulbar invagination.
M.A. (*a*) *and* (*b*)	Branches of the mesencephalic artery. (*Vide A.M.* (*a*) *to* (*c*).)
Med. Obl.	Medulla oblongata.
Med. Sp.	Medulla spinalis.
Mesenceph.	Mesencephalon.
M.C.A. (*a*) *to* (*e*)	Branches of the middle cerebral artery. (*Vide A.C.M.* (*a*) *to* (*e*).)
N. Term.	Nervus terminalis.
N.I. to *N. XII*	Cranial nerves.
Parenceph.	Parencephalon.
Pl. Ch. Vent. IV	Choroid plexus of the fourth ventricle.
S. Amygd. Rud.	Rudimentary amygdaloid sulcus.
S. Cor.	Sulcus coronalis.
S. Endorh. Ext.	External endorhinal sulcus.
S. Endohipp.	Endohippocampal sulcus.
S. Endopal.-Olf.	Endopalaeo-olfactory sulcus.
S. Endopyr. Ant.	Anterior endopyriform sulcus.
S. Endopyr. Post.	Posterior endopyriform sulcus.
S. Lat. Ant.	Anterior lateral sulcus of the medulla oblongata.
S. Lat. Post.	Posterior lateral sulcus of the medulla oblongata.
S. Lob.	Sulcus lobaris.
S. Med. Ant.	Anterior median sulcus.
S. Med. Post.	Posterior median sulcus.
S. Para-Med.	Para-median sulcus.
S. Prim.	Sulcus primus.
S. Septo-Str.	Septo-striate sulcus.
S. Septo-Cort.	Septo-cortical sulcus.
Tect. Mes.	Optic tectum.
V.C. D.-M. Un. Alt.	Alternative mode of union of dorso-medial cerebral veins.
V.D.L. Bifurc.	Bifurcation of the dorsal longitudinal vein.
V.D.L. Div. Term.	Terminal divisions of the dorsal longitudinal vein.
Ven. Anast.	Anastomotic vein.
Ven. Cer. Dors.-Med.	Dorso-medial cerebral vein.
Ven. Cereb. Lat.	Lateral cerebellar vein.
Ven. Cereb. Centr.	Central cerebellar veins.
Ven. Cer. Lat. Ant.	Anterior lateral cerebral veins.
Ven. Cer. Lat. Post.	Posterior lateral cerebral vein.
Ven. Cer. Med. Ant.	Anterior medial cerebral veins.
Ven. Cer. Med. Interm.	Intermediate medial cerebral vein.
Ven. Cer. Med. Post.	Posterior medial cerebral vein.
Ven. Chor. Comm.	Common choroidal vein.
Ven. Chor. Vent. Lat.	Choroidal veins of the lateral ventricle.
Ven. Chor. Vent. IV	Choroidal veins of the fourth ventricle.

Ven. Dienceph.	Diencephalic vein.
Ven. Dors. Long.	Dorsal longitudinal vein.
Ven. Hypothal.	Hypothalamic vein.
Ven. Med. Ant.	Anterior medullary vein.
Ven. Med. Post.	Posterior medullary vein.
Ven. Mes. Ant.	Anterior mesencephalic vein.
Ven. Mes. Ant. and Dienceph.	Anterior mesencephalic and diencephalic vein.
Ven. Med. Bas. Ant.	Anterior basal mesencephalic vein.
Ven. Mes. Bas. Post.	Posterior basal mesencephalic vein.
Ven. Mes. Post.	Posterior mesencephalic vein.
Ven. Mes. Post. and Cer. Centr.	Posterior mesencephalic and central cerebellar vein.
Ven. Olf. Lat.	Lateral olfactory vein.
Ven. Olf. Med.	Medial olfactory vein.
Ven. Sept.	Septal vein.
Ven. Sp. Dors.	Dorsal spinal vein.
Ven. Sp. Ventr.	Ventral spinal vein.
Ven. Tect. Ant.	Anterior tectal vein.
Ven. Tect. Dors. and S.D.	Dorsal tectal veins and tributaries from the dorsal sac.
Ven. Tect. Post.	Posterior tectal vein.
Vent. III	Third ventricle.
Vent. IV	Fourth ventricle.
V.O.L. (*a*) to *V.O.L.* (*c*)	Branches of the lateral olfactory vein.
V.O.M. (*a*) *and V.O.M.* (*b*)	Branches of the medial olfactory vein.

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ANATOMICAL NOTES

A SPECIMEN SHOWING UNUSUAL ARRANGEMENTS OF AUTONOMIC NERVES

By G. A. G. MITCHELL
Department of Anatomy, Aberdeen University

Variations in the arrangement of autonomic nerves are common, doubtless owing to the extensive plexus formations which provide multiple alternative pathways, but most of the anomalies can be classified as being minor rather than major in character. Occasionally, however, a specimen is found which shows radical differences from the usual, and one such specimen is illustrated here. It is a dissection of a male still-born infant, and several features are worthy of notice:

(1) The equality in size of the left thoracic splanchnic nerves.

(2) The direct nerve supply to the left suprarenal gland.

(3) The large direct communication between the right vagus and right greater splanchnic nerves.

(4) The direct communication between branches of the superior hypogastric plexus (presacral nerve) and left genitofemoral nerve.

(5) The unusual plexiform arrangement of the colonic branches arising from the left hypogastric nerve and inferior hypogastric (pelvic) plexus.

(6) The presence of only three lumbar ganglia on the left side.

(1) There are usually three thoracic splanchnic nerves, superior or greater, middle or lesser, and inferior or lowest, and normally they are very unequal in size and do not all terminate in the corresponding coeliac ganglion. In this specimen they were almost equal in size, and they all entered the left extremity of the coeliac plexus directly. The superior (normally the greater splanchnic nerve) arose by three roots from the seventh, eighth and ninth thoracic ganglia, the middle (normally the lesser splanchnic nerve) by two roots from the tenth and eleventh thoracic ganglia, and the inferior (normally the lowest splanchnic nerve) by two roots from the twelfth thoracic ganglion and the part of the sympathetic trunk immediately above it. They all perforated the left crus of the diaphragm close together and then entered the coeliac plexus.

No other example of this arrangement has been found in a series of thirty-two consecutive dissections in which the splanchnic nerves have been examined, nor has it been observed in any ordinary dissecting room subject.

On the right side of this specimen the splanchnic nerves were more unequal in size, but there was less than the usual difference, the lesser and lowest nerves being almost equal in size, though both were smaller than the greater splanchnic nerve which arose from the sixth to the ninth thoracic ganglia inclusive.

(2) On the left side the suprarenal gland received a direct nerve supply from the eleventh thoracic ganglion. This nerve pierced the left crus of the diaphragm above and external to the splanchnic nerves and entered the posterior surface of the suprarenal gland close to its medial border. This nerve has not been discovered in any of the other dissections, and it was absent on the right side of this specimen.

(3) The vagus and thoracic splanchnic nerves always communicate by delicate filaments, usually in the region of the oesophageal plexus, but in two dissections a much larger communication was found between the right vagus and right greater splanchnic nerve. In the specimen shown here the communicating branch can be seen to unite directly with one of the branches of the right vagus, but the point of

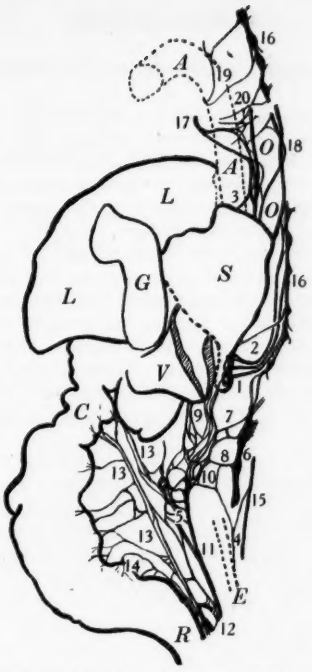

Fig. 1. An outline drawing of a photograph of the actual dissection.

A Aorta.
C Descending colon (drawn over to right side).
E External iliac artery.
G Gall-bladder.
L Liver (left lobe removed).
O Oesophagus (divided at level of aortic arch and displaced to left side).
R Rectum.
S Suprarenal gland (lower half removed: cut edge indicated by dotted line).
V Pyloric end of stomach. The body and fundus have been removed, and the cut edges are indicated by cross hatching.

(1) Terminal parts of left thoracic splanchnic nerves.
(2) Direct nerve supply to left suprarenal gland.
(3) Nerve uniting branch of right vagus to right greater splanchnic nerve.
(4) Nerve formed from branches of superior hypogastric plexus and left genitofemoral nerve.
(5) Plexiform arrangement of colonic nerves.
(6) Lumbar portion of sympathetic gangliated trunk.
(7) Upper lumbar splanchnic nerves.
(8) Lower lumbar splanchnic nerve.
(9) Intermesenteric nerves.
(10) Superior hypogastric plexus (presacral nerve).
(11) Left hypogastric nerve.
(12) Inferior hypogastric plexus (pelvic plexus).
(13) Long colonic nerves arising from inferior hypogastric plexus.
(14) Short colonic nerves arising from inferior hypogastric plexus.
(15) Left genitofemoral nerve.
(16) Thoracic portion of sympathetic gangliated trunk.
(17) Right vagus nerve.
(18) Left vagus nerve.
(19) Thoracic cardiac branches from gangliated sympathetic trunk.
(20) Pulmonary branches from gangliated sympathetic trunk.

junction with the right greater splanchnic nerve is obscured in the drawing by the liver.

(4) It is unusual to find a nerve which is apparently composed of almost equal contributions from somatic and autonomic sources, but in three dissections, including the one at present under discussion, such a nerve has been found. In every case the arrangement has been similar. A filament arising either from the superior hypogastric plexus or from the hypogastric nerve united about the level of the lumbosacral junction with a filament derived from the genitofemoral nerve, and the common nerve so formed ran downwards to supply various structures, such as the ureter, the common and external iliac arteries, and the vas deferens; while in one dissection (not the one illustrated here) it ran behind the external iliac artery for a short distance, supplied a few delicate filaments to it, and then passed downwards to enter the inferior hypogastric (pelvic) plexus. In this specimen the filament from the superior hypogastric plexus was united to the lowest lumbar splanchnic nerve by a vertical twig, a connexion which was absent in the other cases.

This nerve should not be confused with the larger genital and femoral branches of the genitofemoral nerve.

(5) Long colonic nerves have been described arising from the inferior hypogastric plexuses and hypogastric nerves, and in this specimen three such nerves arose directly from the left inferior hypogastric plexus. One of these, as can be seen in the photograph, formed a ladder-like plexiform arrangement with branches from the left hypogastric nerve. Some of the filaments continued upwards to supply the descending colon, while others united with the nerves accompanying the inferior mesenteric artery and were distributed with them.

A somewhat similar arrangement existed on the right side.

(6) Only three lumbar ganglia were present on the left side, and from the size, and the number of rami attached to it, the first ganglion probably represented three fused ganglia. Four ganglia were present on the right side, and this appears to be the commonest number of lumbar ganglia.

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A CASE OF FOETUS WITH ONE EYE (CYCLOPS)

By Prof. M. N. De, M.B., M.R.C.P. (London)
and H. K. Dutta, M.B.

From the Department of Pathology, Medical College, Calcutta

Fig. 1.

It is not uncommon to find imperfections in the development of the face due to imperfect closure of the embryonal clefts, but the fusion of the eyes in the middle line with a rudimentary nose in the forehead above the eye is an anomaly of an extreme degree.

A nearly full-term female foetus, 14 in. in length, shows only one eye situated in the centre of the face. The eyelids do not completely cover the eye, with the result that the greater portion of the bulbar conjunctiva is exposed. The eyebrow is not developed nor is there any evidence of a rudimentary eye-socket on either side of the face.

The shape, size and position of the nose are all abnormal. It is situated in the middle line just above the eye and is a fleshy, tubular outgrowth, about $\frac{3}{4}$ in. in length and $\frac{1}{8}$ in. in diameter with a transversely placed slit in the centre. On microscopic examination all the histological structures of the nose could be found.

The mouth is well formed and the space between the eye and the upper lip which is occupied by the nose in the normal foetus is represented by a broad furrow. The lips and the ears are normal in shape and position.

Inside the skull, some abnormalities could be seen in the development of the first two cranial nerves and the body of the sphenoid bone. Between the two anterior clinoid processes the bone forms a crest and there is no optic groove or pituitary fossa. The olfactory tracts, bulbs and nerves are absent. The two optic nerves emerge from the right and left aspects of the eyeball, pass through the optic foramina, and without crossing or forming a chiasma each continues on its own side.

REVIEWS

The Origin of Life by Continuous Creation. (Die Entstehung des Lebens durch stetige Schöpfung. Von IGNAZ LICHTIG. N.V. Noord-Hollandsche Uitgevers Maatschappij. Amsterdam.) 1938. 7.50 Gulden.

There is, according to the views held by the author, merely a quantitative difference between abiotic and biotic substance, the latter showing a higher degree of complexity. The general evolution of substance reveals the tendency towards progressive complication and integration from electrons to atoms, molecules, ultravisible corpuscles (representing the forerunners of unicellular organisms), and finally the multicellular organisms: the creation of life represents only a transitional stage within the general scheme of material evolution, counterbalanced by the tendency to dissolution of the living substance. The origin of life seems to have taken place about 1500 million years ago for the first time, along the continental coast line. There spontaneous creation continues up to the present time, establishing innumerable patterns of ultravisible organisms and exhausting every possibility of structural form, compatible with the maintenance and proliferation of the entities created. These primitive organisms represent the starting points of parallel phylogenetic lines. A diagram of parallel lines, the longest of which represents the most highly developed organism, Man, should replace the tree diagram representing the monophyletic system which represents the stock of living and prehistoric organisms as having been derived from one single unicellular ancestor. The author emphatically denies the possibility of establishing a tree pattern of general value. Such an attempt to arrange a group of organisms in a definite phylogenetic succession, based upon the progressive transformation of a specific morphological feature, may appear successful, but has to be entirely abandoned when other morphological features of equal taxonomic value are selected for establishing phylogenetic relationships. What appears to be a genetic relationship between the branches of the phylogenetic tree is only the manifestation of innumerable shades of adjustments of the organisms to their surroundings and the innumerable constructive solutions by which the requirements of the basic functions of life can be met. Morphological resemblance does not prove close genetic relationship, but is only the expression of a system of phylogenetic parallels.

These are the principal conceptions of the author. They are based upon a great many observations, arranged in ten large chapters, dealing with the philosophical, biological, morphological, systematical, ontogenetic, palaeontological, phylogenetic, stratigraphical, geographical and palaeoclimatological aspects of the question. It is quite impossible to select, within the framework of a brief summary, any one of the arguments for review. The book, which is written in brilliant German, certainly deserves to be read in full and to be exhaustively discussed. Some of the views held by the author seem to be quite convincing, but many readers will reject, for instance, the purely quantitative conception of life.

A Cerebral Atlas, illustrating the differences between the brains of mentally defective and normal individuals, with a social, mental and neurological record of 120 defectives during life. By RICHARD J. A. BERRY, M.D., F.R.S.E., F.R.C.S.E. (Oxford University Press.) 1938. Pp. xxv+421. 430 photographs. Price £5. 5*s*.

This big atlas contains a photographic comparison of the brains of 120 mental defectives with those of 77 normal people, the former having been obtained from consecutive post-mortem examinations conducted by members of the staff of Stoke

Park Colony. As the mental and other reactions of most of these individuals were known and observed during life, it has been possible to furnish an account of the clinical, neurological and mental phenomena, and to correlate the findings with the brain after death. In an introductory chapter, the author deals chiefly with the technique and the classification employed, and the various measurements and weight of the brain.

The chief differences between normal and defective brains are summarized as follows. In a defective brain there is:

(1) A considerable reduction in size and weight.

(2) A tendency for the persistence in the convolutional pattern of certain prenatal developmental features.

(3) A greater variation than occurs normally in the gyri and sulci bordering the central sulcus of Rolando—that is, in the important effector and receptor areas.

(4) A lack of full development in the opercula, particularly in the frontal one, which frequently fails to cover the insula.

(5) A diminution in the cortical amount of the triangular parietal area—that is, of the area concerned with the association of incoming impulses from the somaesthetic, acoustic, and visual areas.

(6) On the medial surface the corpus callosum appears to be frequently diminished in size, notably the genu and splenium.

(7) The visual areas exhibit more variation in pattern and extent.

(8) Lastly, the sulci generally appear to be shallower in the defective brain than in the normal.

Anatomia dello Scheletro umano fetale. By ROBERTO JONATA. (Bologna: Licinio Cappelli.) 1938. Pp. xix + 285, 128 figs. 235 radiographs, 20 tables. Price 80 lire.

Dr Jonata of the Radiological Institute of Parma has summarized in this book the knowledge of the foetal skeleton acquired by dissection and microscopic observation, and added the results of his researches based on radiological examination. He frankly admits that his own work does little more than confirm and amplify existing knowledge. The usually accepted dates of ossification in the phalanges of the fingers and toes would appear, however, to need slight correction. And he has made the interesting observation that the foetal skull is dolichocephalic for the first two months, becomes brachycephalic during the third and fourth and from this date onwards begins to acquire its individual characteristics. Further, the variations in dates of ossification with sex and bodily length are worth noting by the medico-legal expert.

There are a number of X-ray photographs, an extensive bibliography and a series of tables giving the proportions of ossified and non-ossified tissue in the skeletal elements at different stages.

Dr Jonata has provided a valuable reference book, which should be of use to anyone interested in the foetal skeleton, whether from the anatomical, anthropological, obstetrical or medico-legal point of view.

Practical Anatomy of the Rabbit. By the late B. A. BENSLEY, Ph.D. Revised and edited by E. HORNE CRAIGIE, Ph.D. (Toronto: University of Toronto Press.) 1938. Pp. 320, 91 figs. Price 14*s*.

This is the 6th edition of Bensley's text-book for elementary students, and has been revised and rearranged. The regional sections with explanations and diagrams are good. In the next edition one might suggest a greater precision in some of the statements, e.g. the inferior vena cava is spoken of as "beginning in the *pelvic* cavity" and the portal fissure is defined as "a large depression, accommodating the portal vein at its point of entrance", with no mention of the hepatic artery or bile duct. The index could be improved by some distinction between the principal page references and those which indicate a casual mention. If one looks up "liver", one has to work through ten unimportant allusions to the liver before discovering a description of the organ at the eleventh.

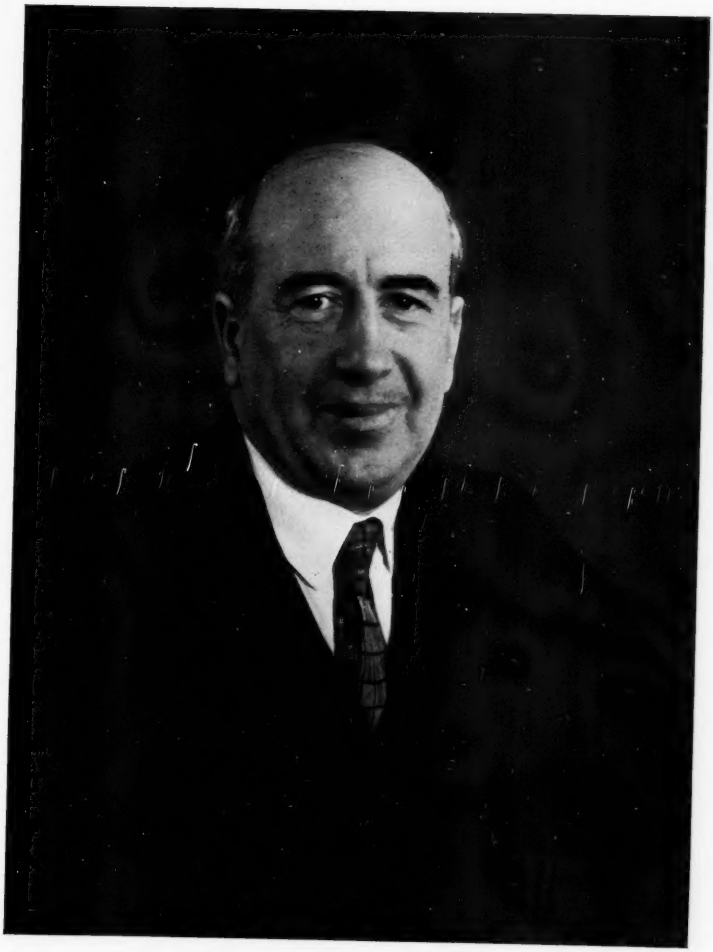
IN MEMORIAM

HERBERT HENRY WOOLLARD

THE sudden death of Prof. Woollard on 18 January at the age of 49 is a serious loss to anatomical science in this country. As successor to the late Sir Grafton Elliot-Smith at University College, London, he was widely recognized as one of the foremost leaders in his subject, and the example of his untiring efforts to promote the progressive development of anatomical teaching and research has for some time been a source of inspiration to many of his colleagues.

Woollard's entry into the field of Anatomy was the result of somewhat fortuitous circumstances. He had qualified in medicine in the University of Melbourne in 1910 and obtained the degree of M.D. in 1912. He took a very active part in the Great War, attaining to the rank of Lieut.-Colonel in the Australian Army Medical Service. There is no doubt that his war experiences imposed a considerable strain on Woollard, leaving a permanent mark on his physical health, and also influencing profoundly his general attitude to life. He was always reluctant in conversation to refer back to these experiences, and to his friends it was evident that he preferred to forget the difficulties with which he had to contend during this time.

After demobilization, Woollard came to London in order to undertake a course of study for the Fellowship of the Royal College of Surgeons, and he elected to do this at University College. We realize now that this was a fortunate choice, seeing that it brought him under the inspiring influence of the late Sir Grafton Elliot-Smith, an influence which was destined to make him completely alter his plans. For, after passing the Primary Fellowship examination, he decided to take up the study of anatomy as a scientific career and, with this end in view, assumed the duties of a demonstrator in the Institute of Anatomy at University College. At this period of his career, Woollard's time was largely taken up with routine teaching. His remarkable capacity for hard work, however, enabled him at the same time to undertake research studies, and he was soon producing original observations of a high order. His earliest work, naturally enough, was directed towards descriptive neurology and comparative anatomy. His first paper—dealing with a particularly difficult problem of the morphology of the reptilian brain—was read at the Summer Meeting of the Anatomical Society at Cambridge in 1920. The present writer well remembers this occasion, since he also read his first paper at the same meeting. We were both raw recruits to anatomy at the time, neither of us understood much of what the other was talking about, and we were both correspondingly impressed. As a matter of fact, Woollard's communication was really quite an outstanding contribution to the anatomy of the reptilian brain, but he never published it *in extenso* because his own critical faculty did not permit



HERBERT HENRY WOOLLARD

UNIV. OF MICH.

him to feel completely satisfied with the evidence which he had collected to establish his points. Indeed, Woollard was never quite happy in regard to the value of purely descriptive anatomy—he realized at a very early stage of his career the vital importance of developing experimental methods for the solution of anatomical problems. This attitude of mind was further strengthened during the year which he spent at the Johns Hopkins School of Anatomy in America as a Fellow of the Rockefeller Foundation.

As a result of his work in the States, Woollard established a considerable reputation for his experimental work, not only in America but also in this country and elsewhere. At this time he completed three important papers. Of these, one (which appeared in the Carnegie Institute Publications) dealt with the development of the limb arteries as demonstrated by the micro-injection of living embryos. This paper—which was beautifully illustrated—threw considerable light on the relation of morphogenetic and haemodynamic factors in the establishment of vascular patterns. Another paper (in collaboration with Wislocki) concerned the phagocytic activity of haemal glands, while the third was a record of experimental observations on the vital staining of the leptomeninges. In the latter, Woollard showed that the mesothelial cells of the pia-arachnoid preserve a generalized character in that they can be transformed under conditions of irritation into freely mobile macrophages—an observation which has an important bearing on the part played by the leptomeninges in resisting infections.

On his return from America, Woollard continued his work at University College. For a time he interested himself in certain problems of a comparative anatomical nature which at the time were interesting students of Primate evolution. In 1925 there appeared from his hand a comprehensive monograph on the anatomy of *Tarsius spectrum*, a monograph which was outstanding for its detailed and critical analysis of the macroscopic and microscopic structure of this remarkable animal, and which had a considerable influence in deciding once and for all its real significance in Primate phylogeny. Other works of a similar type included papers on the cortical architecture of *Tarsius*, the comparative anatomy of the lateral geniculate body in lemurs (in collaboration with Beattie), the brain of *Orycteropus*, the comparative histology of the retina in Primates, and epicritic and dyscritic systems in primitive Primates.

In 1925 Woollard started his work on peripheral innervation. Probably this field of investigation led to his finest achievements in anatomical research. He published papers in 1926 and 1927 on the innervation of the heart, the innervation of blood-vessels, and the structural changes in nerve-endings related to starvation and beri-beri. The success of this work was largely the result of his extraordinary skill with the difficult technique of vital staining with methylene blue.

In 1927, Woollard was appointed Assistant Professor at University College, and in the same year he also published his *Recent Advances in Anatomy*. The influence of this book on the development of anatomy in this country has been

very extensive. It called vigorous attention to the vitality of anatomical science in other countries (particularly the United States), and demonstrated that as a means of approaching problems of structural organization the methods of experimental anatomy offer great opportunities, and indeed, are finally essential.

In 1928, Woollard was appointed to the Chair of Anatomy and Histology in Adelaide, a post which he held for two years. During this comparatively short time, he showed his usual energy in research work, and made good use of the opportunities offered by local material. He made a study of the growth of the Australian brain and its cortical structure, the results of which were published in two papers in the *Journal of Anatomy*; he studied the racial variations in the cutaneous glands of man; and (in collaboration with Cleland) he collected valuable data referring to the distribution of blood groups in Australian aborigines. Woollard returned from Australia to occupy the Chair of Anatomy at St Bartholomew's Hospital. Those of his colleagues who played an unofficial part in urging his qualifications for the post may be excused if they felt a certain gratification when his appointment proved an immense success. For there was a reluctance on the part of some people to believe that a man who had shown himself to be so productive in scientific research would also fulfil the necessary responsibilities of a teacher and administrator. It is worth while drawing attention to this point, for this curious distrust of the successful research worker does tend to manifest itself in appointments of this kind—though happily not to such a degree as formerly. Woollard's contact with his clinical colleagues at Bart's reflected itself in his publications which reveal at once his breadth of interest and his powers of application. He directed his attention particularly to the question of referred pain, the sympathetic system and the anatomical basis of vaso-motor mechanisms. In this work he did very great service to clinical science, particularly in establishing a secure anatomical basis for the surgical treatment of sympathetic disorders and in devising practicable operative approaches for the use of the surgeon. At the same time, Woollard also gained a well-deserved reputation for his teaching and administrative abilities. He was vigorous and stimulating as a lecturer, did much to encourage the personal interest of his students in the development of anatomical science, and planned original and effective changes in the curriculum. In particular, he was always insistent on the fact that (to quote one of the founders of modern histology, Kölliker) "Anatomy is and remains the science of the structural parts of the organism and of the laws of their origin and transformation, whether these structural parts are visible to the naked eye or only through the microscope". He pointed out the utter illogicality of trying to maintain the artificial separation between the teaching of macroscopic and microscopic anatomy, a separation which, in the past, has been simply based on a difference in the technical methods employed. Almost all anatomical problems require for their ultimate solution the application of microscopical technique, and no more serious injury has been done to the progress of ana-

tomical science in this country than the accidental divorce of the systematic teaching of histology from the rest of anatomical science—a divorce which unfortunately is still maintained in some schools. Woollard also emphasized another important reflexion—that histology as a science must inevitably be unproductive and sterile unless it links itself with the more general problems of anatomical science such as morphogenesis, structural organization, and growth processes.

When Woollard finally left St Bartholomew's on an invitation to the Chair of Anatomy at University College (which had become vacant on Elliot-Smith's death), he left behind him a permanent memorial in the magnificent new department of anatomy which he was responsible for planning and equipping. This department not only incorporated much that is new and worthy of note in the teaching accommodation, it also provided the research accommodation which was becoming so urgently necessary under the stimulus of Woollard's directorship.

At University College, Woollard devoted himself to maintaining the fine traditions of the Institute of Anatomy. He developed the teaching on a progressive basis, following naturally on the lines which had already been established by his distinguished predecessor, and rapidly collected around him a band of enthusiastic and devoted workers who engaged themselves in several productive lines of research under his direction and guidance. As regards his own research, he now concentrated his attention mainly on problems of peripheral sensation. He had for some years been using himself as an experimental subject, and aimed at correlating the distribution of different sensory spots in his own skin with different morphological types of nerve-ending. It is a tribute to his enthusiasm for his research that this method of experimentation involved not a little personal discomfort in the numerous small but painful operations which he permitted to be carried out on himself, in order to obtain material for histological study. This work led him to identify specific types of nerve terminal subserving touch and temperature, and he was also able to demonstrate the anatomical basis of Lewis's nocifensor mechanism. At the time of his death, Woollard was pursuing his investigations of these problems by oscillographic methods and was already achieving results of unusual interest. These studies were approaching completion, and it is hoped that his collaborators at University College will shortly be able to assemble their material for publication.

That Woollard's abrupt death at this active stage of his career is something of a disaster for anatomical science in this country is evident enough to his colleagues. He exerted his influence for the progress of his subject by his published work, by the stimulus of his personal contact, by his policy of modifying examinations to bring them into accord with modern developments in the teaching of anatomy, and by his work as Editor of the *Journal of Anatomy*. All these activities involved a very considerable amount of hard work, but he willingly gave much of his time and energy to them, since he felt by doing so

he might be able to raise the status of British anatomy. In fact, his aspirations were already leading to a successful issue, but there remains much more to be done along the same lines. Those of Woollard's colleagues who are actuated by similar motives for the progressive development of anatomical science will find inspiration in his achievements, and will assuredly continue with renewed energy the work which he had begun so well.

Woollard's personal friends will always find singular comfort in looking back on the pleasures of his acquaintance. He was a man of very wide general knowledge and a brilliant conversationalist, and he was gifted with an unusual sense of humour. Consequently, he made an ideal holiday companion. The present writer was fortunate enough to spend several long walking holidays with him, and learnt thoroughly to appreciate his personal attitude towards many of the more ordinary problems and experiences of life. This attitude was essentially generous and always receptive. Although Woollard had earned the reputation among some of his acquaintances of being somewhat uncompromising in his criticisms of other people's work, in actual fact this was only true in reference to work that was hastily and badly done. In such a case, Woollard certainly made no attempt to conceal his criticisms—he was quite intolerant of insincerity and laziness.

Woollard's health during the last two or three years of his life was not good. He himself suspected cardiac trouble because of the acute anginoid pain which attacked him from time to time, but only his most intimate friends were aware of the physical and mental strain which this imposed upon him. His suspicions were amply confirmed at his death, and it now appears remarkable that he was able to achieve so much under such a severe handicap.

By his work and career Woollard had earned a high place among the distinguished anatomists of this country. We deeply regret that he was not permitted to remain longer with us as a colleague and a friend.

W. E. Le G. C.

AN EXPERIMENTAL INVESTIGATION OF THE MOTOR CORTEX AND PYRAMIDAL TRACT OF *ECHIDNA ACULEATA*

By F. GOLDBY
University of Adelaide

The possession of an area of cortex, which on electrical stimulation gives rise to movement in contralateral parts of the body, is well known to be a common and probably universal characteristic of the mammalian forebrain. It is equally well known that this so-called "motor area" contains cells whose axons form a long projection tract, ending, partly in the brain stem and partly in the spinal cord, in immediate or very close relationship with the neurones of the "final common path".

In some of the more primitive mammals, e.g. the American opossum, *Didelphys virginiana*, the excitable area of the cortex lacks clear definition, and will evoke movements only in approximately the forequarters of the body (Gray & Turner, 1924). The cortico-bulbar and cortico-spinal tracts in this and some other mammals have been shown to be small and probably to extend no farther than the upper cervical segments of the spinal cord (Turner, 1924). Further, in modern reptiles, unequivocal evidence for the presence of an excitable cortical area is lacking, and there is no evidence at all for the presence of cortico-bulbar or cortico-spinal tracts.

These observations have made the condition of the surviving monotremes of particular interest. Although highly specialized animals, they retain many reptilian characteristics. One may well enquire, therefore, whether in the brain they have yet developed an excitable motor area, with the corresponding connexions with the brain stem and spinal cord, and if so, whether these structures are even more rudimentary than in primitive metatherian and eutherian mammals.

Although several descriptions, more or less complete, of the appearance and structure of the nervous system of both *Ornithorhynchus* and *Echidna* have appeared, they are nearly all based on the examination of normal material prepared by the usual neuro-histological techniques. Experimental studies have been few, and are in fact limited to two published papers. Martin (1898) published an account of the motor cortex of *Ornithorhynchus*, and Abbie (1938) a more detailed account in both *Ornithorhynchus* and *Echidna*. It is the object of the present communication to place on record the results of some further experiments on the *Echidna*. The work falls into two subdivisions: (i) an examination of the cerebral cortex by the method of electrical stimulation, the results of which will be seen to be, in the main, confirmatory of the work of

Abbie, and (ii) an examination of the connexions of the excitable area by the Marchi technique. It appears that this technique has not before been used successfully on either monotreme.

MATERIAL AND METHODS

(i) *Electrical stimulation of the cerebral cortex*

The apparatus used was that described by Myers (1936) and used by Tower (1936) in her work on the extrapyramidal motor areas of the cat. Alternating current at 50 cycles was drawn from the mains and connected to a transformer with an output at 4, 8 or 16 V. The current from the transformer was allowed to flow continuously through a resistance coil of about 80 ohms, one terminal of which was connected to the indifferent electrode applied to the skin of the animal's abdomen. The stimulating electrode, a platinum wire, was connected to a variable contact on the coils of the resistance, so that the stimulating voltage could be varied at will from 0 to 4, 8 or 16 V.

The operations were all conducted under open ether anaesthesia, the depth of which was just enough to produce moderate relaxation and to abolish the reaction of "rolling up" which is so sensitive in the unanaesthetized animal. The ordinary precautions were taken to avoid injury to the cortex or its blood supply, and the field under investigation was kept moist with warm Ringer's solution, except during actual stimulation.

The cortex was stimulated on one side in fifteen animals. In all but three the primary object of the experiment was the infliction of localized lesions with subsequent study of the resulting degenerations. Except in these three, therefore, only limited areas of cortex were exposed and explored. Results obtained from such limited exposures have a very restricted value, chiefly as confirmatory or otherwise of results obtained when a more complete exposure is possible. The number of hemispheres (three) in which exploration was reasonably complete is, of course, small. The results acquire greater significance, however, since they are consistent with the larger number of incomplete explorations, and with those of Abbie (1938) on eight specimens in which the whole of one or both hemispheres was exposed in each experiment.

The threshold stimulus was measured in volts. It was found that with 1 V., 0·1 mA. flowed through the stimulating circuit, with 2 V., 0·4 mA. Most of the threshold values for the excitable cortex in the different experiments lay between these limits. The apparatus was tested on cats and used in a series of experiments on the phalanger, *Trichosurus vulpecula*. The lowest threshold for the motor cortex of the cat was found to be 0·9 V., 0·1 mA., and for the opossum 1·1 V. and 0·1 mA. In all these experiments the anaesthetic was "open" ether. It is obvious that factors that can be controlled only very imperfectly or not at all, such as the depth of anaesthesia, the general condition of the animal and so on, may lead to considerable variations in the threshold value obtained in any individual experiment. The figures therefore can have

little value except as indicating that the threshold for the motor cortex of three very different mammals is in each case of the same order of magnitude. The actual figures obtained are closer than one would have expected.

(ii) *Degeneration experiments*

In all, ten experiments were performed with the object of tracing efferent fibres from the cortex by the Marchi method, but only one of these has proved successful. Of the ten, two died under the anaesthetic, and four survived the operation by less than 10 days. Of the remaining four, one died on the 27th day after operation, and when found was too decomposed to be of any value. Sections were prepared from three specimens: *Echidna* XIV, which died on the 13th day after operation, and was fixed within an hour of death, *Echidna* II, killed on the 28th day, and *Echidna* XIII, on the 44th day after operation.

Two techniques were used in the preparation of the material. One was the ordinary Marchi technique with preliminary fixation in 10 % formalin, followed by 10 days' treatment in 3 % potassium bichromate, and finally by 21 days in the osmic acid-bichromate mixture. The other was the modification described by Swank & Davenport (1935). Here potassium bichromate is not used (it is replaced by potassium chlorate), so that the tissue does not become brittle and can be cut more easily. In addition artefact formation is less, and the sections will take a good counterstain with cresyl violet. This modification was tried in a preliminary experiment on a cat, and gave very satisfactory results.

In each specimen the whole brain was cut into slices 5 mm. or less in thickness, and similar slices were taken from three levels of the spinal cord: lower cervical, mid-thoracic and from the lumbo-sacral enlargement. The blocks were embedded in celloidin or paraffin, and as a general rule every fifth section mounted, in some regions more, in others less. The paraffin blocks were cut at 20 μ, the celloidin at 40 μ.

Echidna XIV, treated by the Swank & Davenport modification and cut in celloidin, gave entirely negative results. It had survived an extensive cortical lesion only 13 days. A serious disadvantage of the technique showed itself in this specimen, namely, patchy but intense staining of normal myelin sheaths. This can be avoided, according to the authors, by modifying the technique in certain directions to suit the material.

Echidna II, killed 28 days after operation, was treated in the same way, but cut in paraffin. Paraffin is not recommended by Swank & Davenport, but I had found it satisfactory in the trial experiment on the cat. It is of course much more convenient. Again the result was entirely negative.

In *Echidna* XIII, killed on the 44th day from operation, alternate blocks were treated by the Swank & Davenport modification and by the ordinary Marchi technique outlined above; paraffin embedding was used for the former and celloidin for the latter on account of the brittleness of the tissue. Every block on which the Swank & Davenport modification was used showed no

osmic acid staining; all the others showed unequivocal positive results which are reported in the later part of this communication. The extent of the successful blocks is shown by the stippled bands in Fig. 2. It was fortunate that they included the crucial regions of the lesion itself, with the formation of the cerebral peduncle, the level of the decussation, and the transition from brain stem to spinal cord.

It is probable that the failure of the Swank & Davenport method was due to the solubility of the products of degeneration in cedar wood oil and hot paraffin, and that the success in the preliminary experiment on a cat was an unfortunate accident. Similar results have been obtained in a parallel series of experiments on the phalanger, *Trichosurus vulpecula*. Potassium bichromate inhibits this solubility, but makes celloidin embedding advisable on account of its hardening action. The main disadvantage of the Swank & Davenport method, its tendency to stain normal fibres, could probably be overcome by experience. When the experimental animal is of an unusual type and can be obtained only in small numbers, it would seem best to keep to the standard Marchi technique.

Since *Echidna* II was embedded in paraffin, its negative evidence is of no value. With respect to the time necessary to produce degenerative changes that can be stained, it can be said only that it is longer than 13 and shorter than 44 days. It is probable that the longer period is advisable, although it adds considerably to the difficulty of the work on account of the heavy post-operative mortality.

The material is adequate for the study of the course of a long projection tract, extending throughout most or a considerable part of the nervous system. It is not adequate for the detailed study of short fibres intrinsic in the forebrain. Such evidence as could be found concerning the latter will be reported, but it must of necessity be incomplete. The usual criteria have been used in assessing the sections. Staining has been accepted as evidence of degeneration resulting from the lesion only when it was unilateral and consistently present throughout a series of sections. On these grounds, and allowing for the gaps in the series, it may be taken that positive evidence is reliable, but that negative evidence has comparatively little value. Artefact formation was not very conspicuous, especially in the forebrain and brain stem. It was more marked in the spinal cord, and particularly in the spinal nerve roots, which would have been subjected to some stretching and other minor trauma in removing the cord.

ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX

In all the earlier experiments only the anterior half of one hemisphere was exposed, i.e. cortex which lay anterior to sulcus β. This region was inexcitable except with strong stimuli varying from 3 to 5 V. The results of such strong stimulation were fairly uniform. Over the whole area, but more marked posteriorly and medially than elsewhere, they were retraction of the head,

accompanied by erection or depression of the spines over a transverse band about the level of the forelimbs. The erection of the spines was sometimes more marked on the heterolateral side. The only other movement seen in these experiments was obtained more easily over the anterior part of the frontal lobe and consisted of a depression of the snout with rotation of the head in one direction or the other, occasionally with slight movements of the jaws. In experiments in which nearly the whole hemisphere was exposed, these movements could be obtained on strong stimulation from practically the whole dorsal and lateral surface. They were also obtained from a necrotic area of cortex in the frontal lobe of an animal, anaesthetized with "dial", that had been operated on four weeks previously. There is therefore no doubt that they are due to the spread of current beyond the cortex, probably to subcortical structures. They were not obtained by stimulating the dura, skull or other adjacent non-nervous structures. They could be obtained at a slightly lower threshold from the subcortical white matter.

Between the sulci α and β quite different results were obtained. The threshold stimulus in the three animals where exposure was nearly complete, was 1·5 V. in one and 1·1 V. in the two others. The excitable area between these sulci fell short of the mid-dorsal line by about 1 cm. and extended downwards to about the level of the pseudo-sylvian fissure. Cortex below this level was not exposed. The order and extent of the representation of movements of different parts of the body were found to be the same as those described and figured by Abbie (1938). The area from which movements of the hindlimbs were obtained, extended posterior to the upper part of sulcus α in one case. In another, the whole anterior boundary of the excitable area fell short of sulcus β by about 1 mm., a finding easily explicable in terms of the variability of this sulcus.

The movements themselves, not very accurately localized within the area in question, were often difficult and sometimes impossible to separate from one another. Movements of the tail (seen in only two animals), were combined with hindlimb movements; movements of the trunk with those of the limbs, usually the hindlimbs; and of the head and neck with those of the forelimbs. By keeping strictly to a threshold stimulus one could nearly always isolate movements of either fore- or hindlimb, but only a very slight increase in the strength of stimulus led to extension of the movement either to the trunk or to the other limb. The hindlimb movements were constantly heterolateral, as also were the few movements of the tail and trunk. The forelimb movements showed a marked tendency to be bilateral, but were always more pronounced on, and sometimes confined to, the heterolateral side.

The movements obtained from the hindlimbs were varied. Extension of the whole limb was seen most often, sometimes combined with abduction and medial rotation. Flexion of the limb was seen less often. In two cases extension was limited to the ankle joint and digits, and in one to the enlarged second digit alone (cf. Abbie, 1938, p. 148).

In the forelimbs two types of movement were seen with about equal frequency: a flexion forwards of the shoulder, lifting the whole limb; or a complex movement consisting of weak extension of the limb combined with medial rotation and abduction. Isolated movements of the digits were not seen in the forelimb.

The movements of the tail were simple elevation or flexion to the opposite side; of the trunk, flexion, with the concavity towards the opposite side; and of the head and neck, elevation or flexion to one side or the other.

This description is based mainly on the three animals in which the cortical exposure was most complete. In eight other experiments, in which limited exposures were made of parts of the cortex outside the area between the sulci α and β, the results of stimulation were uniformly negative, except with the high voltages already mentioned. Four others, with limited exposures including part of the area between the sulci α and β, all gave positive results. Hindlimb movements were obtained most often because the upper part of the excitable area is more easily accessible. In two of these high thresholds were observed, 2·5 and 7 V. respectively. It is probable that 2·5 V. is within the limits of experimental error, and it may have been due to greater depth of anaesthesia, but it is difficult to account similarly for the very high reading of 7 V.

In this series of experiments all regions of the cortex have been explored except for the posterior and ventral part of the temporal lobe, the temporal lobe below the level of the pseudo-sylvian fissure, and the whole medial surface of the hemisphere. It must be remembered too, that many of the sulci are very deep, so that a considerable area of cortex lies below the surface and is inaccessible to direct stimulation. No movements of the eyelids, jaws or tongue were obtained from the cortex in any experiment where the probability of spread of stimulus beyond the cortex could be excluded. This finding must be accepted only in relation to the limitations just mentioned.

It is clear that these results differ only in detail from those of Abbie (1938). They appear to show, for example, some predominance of extensor movements, and also of complex movements involving several segments of a limb. A predominance of extensor movements may be of significance in view of the fact that the normal resting posture of this animal is one of general flexion. Taken generally, however, differences are of a minor character and probably the result of unimportant variations in technique. One may conclude, therefore, that the *Echidna* possesses an electrically excitable "motor area" of cortex, the threshold stimulus for which is of the same order as that for the motor area of other mammals. The area as a whole can be defined with considerable precision; there is an ill-defined localization within it for movements of the tail, hindlimb, trunk, forelimb and head, in that order, from above downwards. The regions from which these different movements can be elicited overlap considerably. The completeness with which movements of nearly the whole body are represented is remarkable, but the variety of movement is not great. These findings

differ from those in many primitive mammals; the most important points of difference are: (i) the completeness with which the whole body is represented, and (ii) the fact that the hindlimbs have at least as extensive and detailed a representation as the forelimbs.

FIBRE DEGENERATIONS FOLLOWING INJURY TO THE MOTOR CORTEX

The superficial extent of the cortical injury in *Echidna* XIII can be seen in Figs. 1 and 2. It occupies that part of the cortex between sulci α and β from which movements of the hindlimb and tail can be elicited, and encroaches on the region for movements of the trunk and forelimb. At the operation the region in question was identified by electrical stimulation, and shown to yield movements of both hind- and forelimb. In Figs. 4 and 5 the depth of the lesion is shown. It has opened the lateral ventricle and caused some damage to the alveus fibres of the hippocampus in its ventro-medial wall. There is no injury to the corpus striatum or other basal structures.

The following degenerations could be observed in the stained sections:

(i) *Association fibres*

A very fine diffuse degeneration in the deepest layers of the cortex and in the white matter immediately subjacent could be seen surrounding the lesion on all sides; medially it extended from the lesion into the medial wall of the hemisphere, and laterally and ventrally for between 1 and 2 mm. from the lesion (Fig. 4). Anteriorly, it could be followed a little beyond the level of Fig. 3 (see Fig. 2). This anterior extension is narrow transversely. It does not reach to the dorso-medial border of the hemisphere, and extends very little further ventrally than the level of the lower border of the lesion (Fig. 3). It can be distinguished from degeneration in the external capsule, being more superficial and generally of a finer and more diffuse nature. Behind the lesion it spreads out on the dorsal and medial aspects of the occipital lobe, and in this situation is more pronounced, containing a fair number of coarse granules.

This superficial degeneration seems to indicate the presence of association fibres, running predominantly in an antero-posterior direction. The coarser degeneration in the occipital lobe may be the result of damage to some of the fibres of the optic radiations. On account of the gaps in the series no more definite statement can be made about their distribution.

(ii) *The external capsule*

Degenerated fibres can be seen in the posterior 1/2 or 2/3 of the external capsule (they are shown in Figs. 3, 4 and 5). Many of these fibres run ventrally and anteriorly to enter the posterior part of the anterior commissure. They cross to the opposite hemisphere and are distributed to an area of cortex corresponding approximately to the lesion, but rather more extensive. Whether some have any other destination could not be determined from this material.

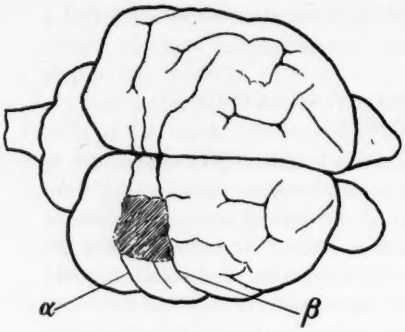


Fig. 1.

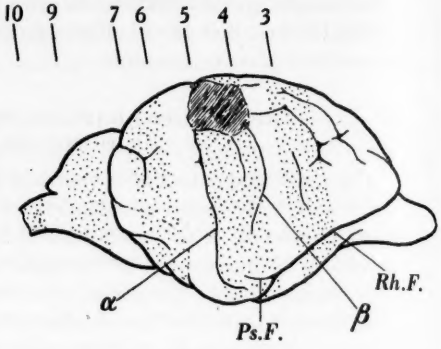


Fig. 2.

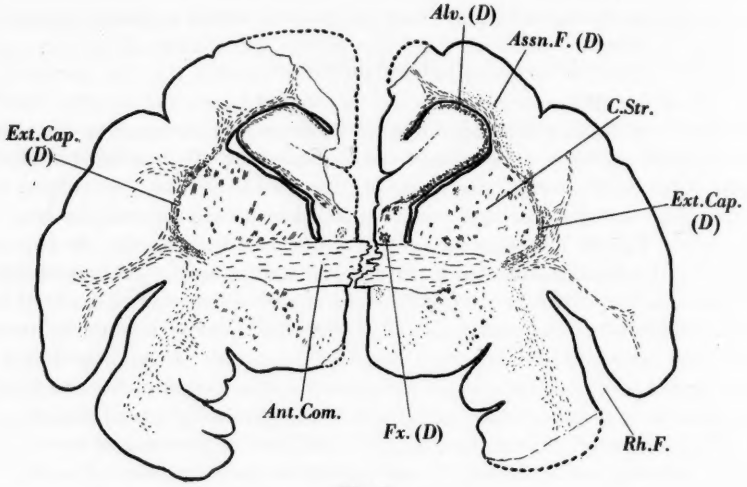


Fig. 3.

Fig. 1. A dorsal view of the brain of *Echidna* XIII, drawn with the camera lucida, to show the position of the lesion. About natural size. The lettering and terminology of the sulci and fissures in this and subsequent figures are taken from Elliot Smith (1902); only those that are relevant to the description in the text are marked.

Fig. 2. A right lateral view of the brain of *Echidna* XIII drawn with the camera lucida and about natural size. The lesion is obliquely shaded. The stippled bands show the position of the blocks in which Marchi staining was satisfactory (see text, p. 512). The oblique lines above the figure show the approximate levels from which Figs. 3–9 are drawn.

Fig. 3. A transverse section about the middle of the anterior commissure (× 2·5). This and other sections were drawn with the Edinger projection apparatus. In some places, owing to the buckling of the thin slices of brain, or to breaking of the very brittle tissue, the sections are not quite complete. Where this is so, the outline has been filled in with an interrupted line.

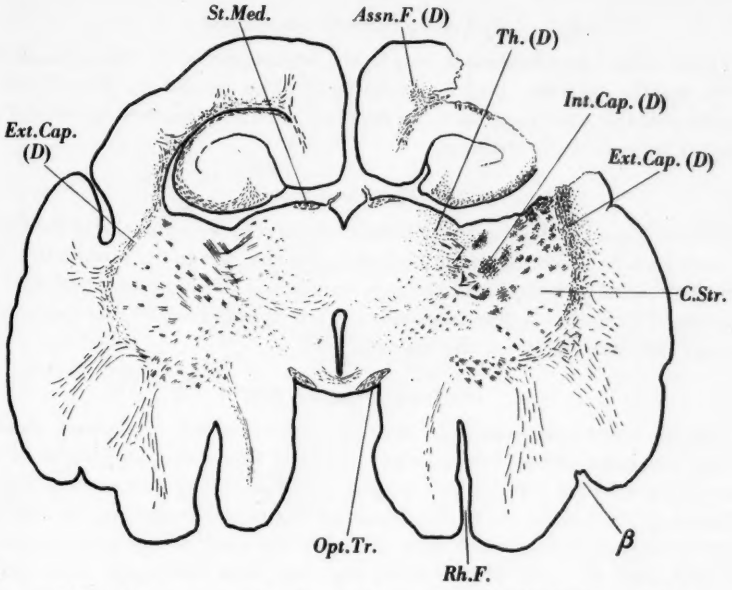


Fig. 4. A transverse section through the anterior part of the lesion. ×2·5.

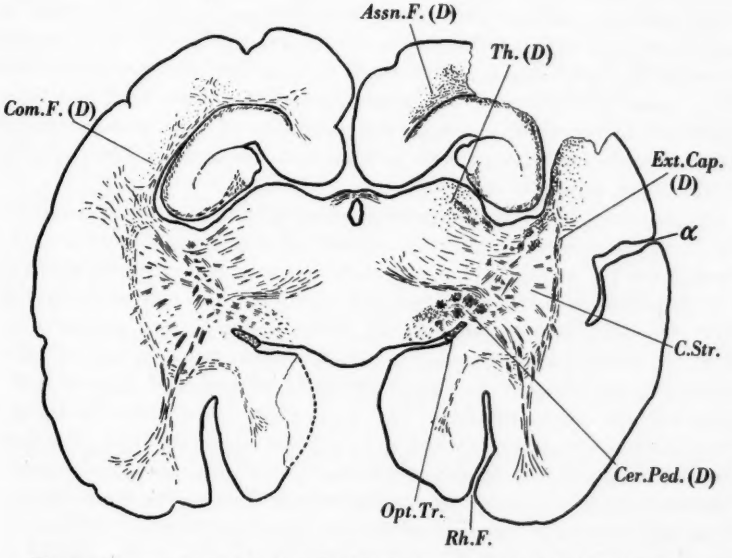


Fig. 5. A transverse section through the posterior part of the lesion and showing the beginning of the cerebral peduncle. ×2·5.

(iii) *Cortico-thalamic connexions*

Fine diffuse degeneration is seen in the lateral part of the thalamus and in some bundles of fibres that are entering it (Figs. 4 and 5). From Abbie's figures (1934) it would appear to be confined mainly to his pars lateralis of the ventral nucleus of the thalamus.

(iv) *The alveus system*

There is marked degeneration in the alveus and fimbria anterior to the lesion. Posterior to the lesion degeneration is insignificant. From the fimbria degenerated fibres can be traced into the precommissural fornix system and into the column of the fornix on the same side. A few appear to cross and can be seen among the alveus fibres of the opposite side.

(v) *Long projection fibres*

As in many mammals, the internal capsule is not a compact mass of fibres, but is broken up into a large number of fascicles of varying sizes that run independently through the corpus striatum. They are seen in Fig. 3, anterior to the lesion, where they show no degenerative changes. In Fig. 4, a section through the anterior part of the lesion, they are larger, and many, coming from the part of the cortex that has been destroyed, show intense degeneration. In Fig. 5, near the posterior border of the lesion, they can be seen again, joining the beginning of the cerebral peduncle. Others, still in the corpus striatum, are more compactly arranged here, so that they form an ill-defined internal capsule between the corpus striatum and the thalamus. It is probable that more degeneration should be seen here than is shown, but there was a small patch, just in this region, where the osmic acid had failed to penetrate. The continuity of degenerated bundles of fibres through the corpus striatum could easily be traced in sections from the anterior part of this block, of which Fig. 4 is an example.

Degenerated fibres in the cerebral peduncle in the mid-brain are shown in Fig. 6, at the level of the inferior corpora quadrigemina. They are scattered throughout the peduncle, but are more numerous towards its medial side. A little posterior to this level, but still anterior to the root of the trigeminal nerve, the decussation begins (Fig. 7). It lies mainly in that part of the pons, named by Elliot Smith, the rostrum, which projects forwards between the roots of the two trigeminal nerves. The interlacing bundles of normal and degenerated fibres are very clearly seen here. This decussation is surrounded by the cells of the pontine nuclei, a few of which are scattered among its fibres. The decussation and the pontine nuclei are covered superficially by a sheath of transversely directed fibres in which no degeneration occurs. These fibres can be traced into the middle cerebellar peduncle which runs into the cerebellum entirely behind the trigeminal nerve root. The distribution of the cells of the pontine nuclei and the destination of the superficial transverse pontine fibres were observed in

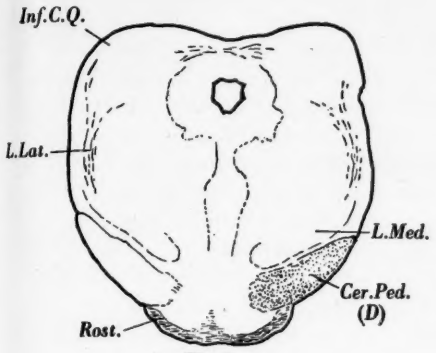


Fig. 6.

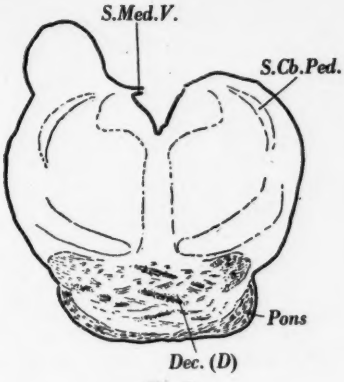


Fig. 7

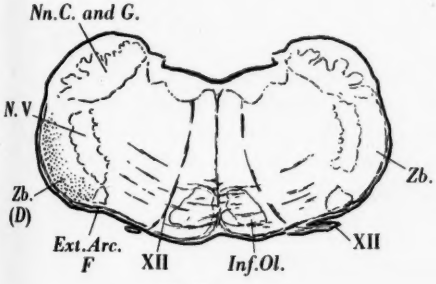


Fig. 8.

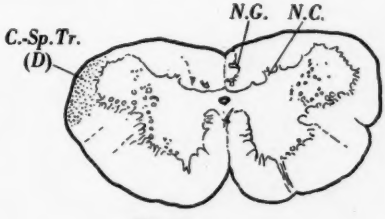


Fig. 9.

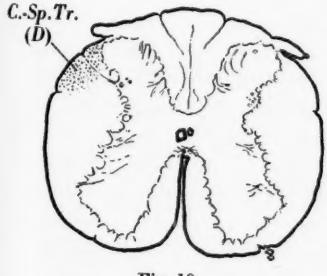


Fig. 10.

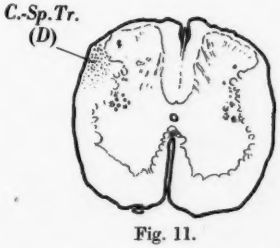


Fig. 11.

Fig. 6. A section through the inferior corpora quadrigemina and the rostrum of the pons. ×5.
Fig. 7. A section through the pontine decussation of the cortico-spinal tract. ×5.
Fig. 8. A section through the medulla, showing some rootlets of the hypoglossal nerve, and the degeneration of cortico-spinal fibres in the "Zonalbündel". ×5.
Fig. 9. A section at the level of the foramen magnum. ×7·5.
Fig. 10. A section through the 7th cervical segment of the spinal cord. ×7·5.
Fig. 11. A section through the 24th segment of the spinal cord. ×7·5.

two series of sections, transverse and sagittal, of the normal brain stem. The sections were stained alternately by toluidine blue and by Weil's modification of the Weigert technique.

The decussation appears to be complete, and posterior to the pons degenerated fibres can be traced on the opposite side in the "Zonalbündel" of Kölliker (1901) or the "tractus temporo-trigeminalis" of Abbie (1934). They are shown here in Fig. 8 at the level of the exit of the hypoglossal nerve. They lie external to the fibres of the spinal root of the trigeminal nerve and are covered by the external arcuate fibres. There is some intermingling of degenerated fibres with those of the spinal root of the trigeminal nerve.

At the junction between the brain stem and the spinal cord the degenerated tract occupies a similar position, in close relationship with what is now the posterior horn of grey matter (Fig. 9). Its fibres are beginning to enter the lateral columns of the spinal cord and are shown in their final position in Fig. 10. They lie quite superficially in the most posterior part of the lateral column, and at this level (the 7th cervical segment) the tract is limited anteriorly by a longitudinal groove on the surface of the cord. The degenerated tract was seen in the same position in the 6th thoracic segment and again in the lumbo-sacral enlargement (Fig. 11). In this part of the cord it is smaller and less clearly defined. This last section was taken from the level of the 24th spinal nerve root. The *Echidna* has seven cervical, sixteen thoracic and two or three lumbar vertebrae, so that in terms of vertebrae the section corresponds to the 16th thoracic. No sections were cut behind this level.

DISCUSSION

The results of the degeneration experiment fall into two categories, (i) those which concern intrinsic fibres of the forebrain, and (ii) those concerning projection fibres which run to the brain stem and spinal cord. Results in the second category have a closer relation to the stimulation experiments and form the main subject of this communication. They will be considered first.

The cerebral injury involved an area of cortex from which movements of both fore- and hindlimbs were elicited by electrical stimulation. The most obvious interpretation of the degeneration that resulted, is to say that the injury has destroyed certain neurones in this cortical area, the axons of which form a long cortico-spinal tract; and that, although this tract differs in its position in the brain stem and in the level at which it decussates, it represents the pyramidal tract of other mammals. It is particularly well developed in the *Echidna*, reaching at least as far down as the 24th segment of the spinal cord. Its extent is indeed its most surprising feature, but is consistent with the observation of Abbie that movements of the tail and hindlimbs can be observed on stimulating the appropriate cortical area, and that they are abolished after section of the lower thoracic spinal cord.

Several views have been expressed concerning the position of the pyramidal tract and its decussation in the *Echidna*, all based on normal histological

material. These experiments confirm the description given by Fuse (1926*a*), and more recently by Addens & Kurotsu (1936) and Yamada (1938), all of whom state that Kölliker's "Zonalbündel" is a laterally situated pyramidal tract which has decussated in the pons. Fuse (1926*c*) classified it as his second or mesencephalic type of decussation. Kölliker's original suggestion, that the "Zonalbündel" is an ascending sensory tract, is therefore wrong, and Abbie's term for it, the "tractus temporo-trigeminalis", is inappropriate. They do not support the tentative suggestion put forward by Ziehen (1897 and 1908) and later amplified by Abbie (1934), that the pyramidal tract is small, and situated on the ventral surface of the medulla "in the position characteristic of all mammals" (Abbie). Neither do they support the suggestion put forward by Kölliker & Ziehen, and reported by Abbie as a definite finding, that the decussating fibres seen in the antero-median sulcus in the region of junction between the medulla and spinal cord, are pyramidal in nature.

The interpretation given above is no doubt in the main correct, but certain reservations must be made. In the first place the cortical injury was deep and destroyed the subjacent white matter down to the ventricle. Although in view of the results of electrical stimulation it is highly probable that the degenerated tract arose in the cortical area destroyed, the degeneration experiment alone does not prove it. It might have arisen in some other part of the cortex and have been interrupted as fibres of passage in the sub-cortical white matter. This is not very likely, as cortical projection fibres in general seem to take a short and direct course before they enter the corpus striatum, which was not injured.

Secondly, it was only the area of cortex concerned in movements of the forelimbs and parts of the body posterior to them that was damaged. The area for movements of the head and neck lies more laterally and ventrally and its projection fibres appear to run straight into the corpus striatum and to have escaped injury. It is probable that not the whole pyramidal tract but only its cortico-spinal part was damaged, and the resulting degeneration affects exclusively that part. It is therefore possible that cortico-bulbar fibres take a different course in the brain stem, in the position, for example, that Abbie (1934) described for the pyramidal tract. Provided they were few in number, or non-myelinated, they might also be accompanied by cortico-spinal fibres, the whole pyramidal system being duplicated as Addens & Kurotsu suggest. It is relevant to say here that Yamada (1938) has been unable to confirm in normal material the presence of the ventrally situated pyramidal tract of Abbie and of Addens & Kurotsu. The last two authors themselves were unable to trace this ill-defined tract cranially into the forebrain or caudally into the spinal cord.

It may be concluded, finally, that a well developed myelinated cerebro-spinal tract exists in the *Echidna*, and that it almost certainly arises from the cerebral cortex between the sulci α and β. After a short course in the cerebral peduncle it decussates completely in the upper part of the pons. It then runs caudally in Kölliker's "Zonalbündel", and is continued in the lateral columns of the spinal cord at least as far as the 24th spinal segment. In view of the doubt

that exists about the interpretation, from normal material, of a small and ill-defined tract close to the mid-ventral line of the medulla, it would be inadvisable to postulate a duplication of the pyramidal tract or a separate course for the cortico-bulbar fibres. The final interpretation of this tract will depend on experimental investigation. This will probably be difficult, as the tract in question is small, consists at best of finely myelinated fibres, and is perhaps predominantly non-myelinated.

It is not intended here to discuss at any length the theoretical implications of these findings. It is of interest to note, however, that a high decussation of the pyramidal tract is particularly characteristic of a small number of highly specialized mammals, all probably of great phylogenetic age. Fuse (1926*b*), among others, has described a decussation just caudal to the pons in some bats and edentates. There is a tendency in some of them for fibres from this high decussation to take up a lateral position in the medulla, e.g. in an armadillo, *Lysiurus unicinctus*, and the pangolin, *Manis tricuspis* (Fuse, 1926*b*). In none is the decussation so high as in the *Echidna*, nor does the tract, after decussation, lie in such an extreme lateral position. It is unfortunate that clear information about the condition in *Ornithorhynchus* is lacking.

On the basis of these findings one may suggest that the earliest mammals possessed no more than a common tendency to develop a motor cortex and a pyramidal tract, and that this tendency has been realized in divergent primitive groups, independently, and in slightly different ways. Chiroptera, Edentata and Monotremata are all highly specialized, but probably developed their specializations at a very early period in mammalian evolution. They have escaped extinction by taking to the air, by covering themselves with armour or spines, or by a fortuitous isolation in inaccessible parts of the world. In them, perhaps, are preserved some early experiments in the development of a pyramidal tract, dating from a time when the mammalian nervous system was less stable than it is now.

The degeneration of fibres, intrinsic in the forebrain, needs little discussion. Although adequate for the elucidation of the main facts about the course of a long fibre tract extending throughout practically the whole nervous system, the material is not suitable for investigating the detailed distribution of comparatively short fibres. The degeneration in association fibres, in the anterior commissure, and in the alveus and fornix systems is such as would be expected from the nature of the lesion. There is nothing unexpected in it, and it brings to light no new facts of importance. In considering the cortico-thalamic degeneration it must be remembered that the time allowed between the operation and the killing of the animal was long, 44 days. This would be quite long enough in many mammals for retrograde degenerative changes to make their appearance in thalamo-cortical fibres. The degeneration seen here cannot therefore be taken as evidence determining the normal direction of conduction in these fibres. No more can be said than that they run between the cortex and the thalamus.

SUMMARY

A description has been given of the results of the electrical stimulation of the cortex of a number of specimens of *Echidna aculeata*.

The results in the main confirm those of Abbie (1938). An excitable area, far back between the sulci α and β, is present. In it, movements of the tail, hindlimb, trunk, forelimb and head and neck are represented, in that order, from above downwards. The threshold for effective stimulation is of the same order of magnitude as that for the motor cortex of other mammals.

Destruction of the upper part of this area leads to degeneration in a long cortico-spinal tract which has been demonstrated by the Marchi method. The tract runs through the cerebral peduncle, decussates in the pons, and continues in the "Zonalbündel" of Kölliker, superficial to the spinal root of the trigeminal nerve throughout the medulla. It enters the most posterior part of the lateral column of the spinal cord and has been traced as far caudally as the 24th spinal segment. No evidence was found for the presence of a pyramidal tract close to the mid-ventral line of the medulla, nor for a decussation in the usual position at the caudal end of the medulla.

Degeneration in intrinsic fibres of the forebrain, resulting from the same lesion, is also described. It affects association fibres, the anterior commissure, cortico-thalamic connexions and the alveus and fornix systems, but brings to light no new facts of importance.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the willing assistance and co-operation of two senior students in the Anatomy Department of the University of Adelaide, who have been acting as part-time technicians. Mr A. D. Packer has assisted at all stages of the technical processes involved, and Mr I. G. Jarrett has been responsible for the preparation of most of the series of sections. I also have to thank the staff of the Physics Department for their help in making and calibrating the apparatus used for electrical stimulation.

ABBREVIATIONS

N.B. The letter (D) placed after any abbreviation indicates that the structure in question shows degenerative changes.

Alv.	Alveus	*Fx.*	Fornix
Ant.Com.	Anterior commissure	*Inf.C.Q.*	Inferior corpus quadrigeminum
Assn.F.	Association fibres	*Inf.Ol.*	Inferior olive
C.-Sp.Tr.	Cortico-spinal tract	*Int.Cap.*	Internal capsule
C.Str.	Corpus striatum	*L.Lat.*	Lemniscus lateralis
Cer.Ped.	Cerebral peduncle	*L.Med.*	Lemniscus medialis
Com.F.	Commissural fibres	*N.C.*	Nucleus cuneatus
Dec.	Decussation	*N.G.*	Nucleus gracilis
Ext.Arc.F.	External arcuate fibres	*N.V.*	Nucleus of the spinal root of the trigeminal nerve
Ext.Cap.	External capsule		

Opt.Tr.	Optic tract	*S.Med.V.*	Superior medullary velum
Ps.F.	Pseudo-sylvian fissure	*St.Med.*	Stria medullaris
Rh.F.	Rhinal fissure	*Th.*	Thalamus
Rost.	Rostrum of pons	*Zb.*	"Zonalbündel"
S.Cb.Ped.	Superior cerebellar peduncle		

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A SPECIFIC FACTOR IN EXTRACTS OF THE CHOROID PLEXUS INFLUENCING THE PERMEABILITY OF THE MYELENCEPHALIC ROOF

By HENRY COHEN and SARAH DAVIES

From the Department of Medicine, University of Liverpool

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I. INTRODUCTION

Previous investigations (Cohen & Davies, 1937, 1938) have established a correlation between the first extraventricular spread of fluids injected into the central nervous system of various embryonic types and the development of the choroid plexus. Two membranous areas in the roof of the fourth ventricle, one anterior and the other posterior, served as the sites of fluid escape. In chick embryos the spread of cerebrospinal fluid from the neural canal to the periaxial spaces was found to be coincident with the development of the choroid plexus. In rabbit, guinea-pig and rat embryos the extracerebral spread of cerebrospinal fluid could be induced when a hypertonic saline solution was injected into the blood stream, but only after the choroid plexus was differentiated.

The present research was undertaken with the object of determining the mechanism underlying the first extracerebral spread of fluid and affecting the permeability of the specialized areas described in our earlier papers. After preliminary experiments, it was considered that the problem should be

investigated, firstly by ascertaining whether or not a factor is present in the choroid plexus which influences tissue permeability; and, secondly, by attempting to induce an extracerebral spread of fluids injected into the embryonic central nervous system at stages prior to the development of the choroid plexus.

II. SURVEY OF LITERATURE

In recent years many investigations have been carried out on the influence of extracts of different mammalian organs on tissue permeability. This work has been reviewed by McClean (1933) and Duran-Reynals (1936). Of particular interest are the observations recorded by McClean (1930, 1931), Hoffman & Duran-Reynals (1931) and Claude & Duran-Reynals (1934).

McClean (1930), investigating the effect of aqueous extracts of mammalian testis on the dermal response to vaccine virus, concluded that the activity of the extract was associated with an increase in dermal permeability. A further investigation (McClean, 1931) showed that the extract caused swelling and distortion of the fibre bundles of the dermis.

Hoffman & Duran-Reynals (1931) investigated the influence of testis extract on the intradermal spread of injected fluids. Using either India ink or a double solution of potassium ferrocyanide and iron ammonium citrate, later precipitated as Prussian blue, these workers found that the extract produced a more extensive spread of the inert particles through the intercellular spaces than did the control suspension made with Ringer's solution. Extracts of kidney possessed a similar property but to a lesser degree. Spleen extracts and blood serum, on the other hand, inhibited the intradermal spreading of the dyes. Hoffman & Duran-Reynals suggested, therefore, that testis extract has the property of altering the permeability of tissue cells and thereby increasing the spread of injected materials, both soluble and particulate.

Claude & Duran-Reynals (1934) extended their investigations to organs other than mammalian testis. Intradermal injections of different organ extracts plus India ink were made in the flank of adult rabbits. The authors showed that many organs possess the power in varying degrees of increasing tissue permeability, but that their potency was always less than that of testis extract. In the light of our own investigations the observations recorded on brain extract are of particular interest. Of ten tests made with extract of rabbit brain, 90 % yielded a positive result with increased diffusion of the dye. The average ratio of spread of the brain extract to the spread of the control expressed in terms of area was 1·9 : 1.

The authors give no indication of the portion of the brain from which the extract was prepared. They merely state that the extract of an entire organ was used in each test.

A search of the literature has revealed no record of investigations concerning the choroid plexus in relation to tissue permeability.

III. THE EFFECT OF EXTRACTS OF CHOROID PLEXUS ON DERMAL PERMEABILITY

(*a*) *Method of investigation*

In investigating the effect of choroid plexus extract on dermal permeability the following series of injections were made:

Series 1. Extracts of rabbit, sheep, cow and dog choroid plexus were injected into rabbits and, for purposes of comparison, extracts of rabbit meninges, brain and blood serum.

Series 2. Extracts of rabbit, sheep, cow and dog choroid plexus were injected into guinea-pigs.

Series 3. Extracts of dog, rabbit, sheep and cow choroid plexus were injected into dogs.

Series 4. Extracts of rabbit choroid plexus and of rabbit testis were injected into rabbits, both extracts being injected separately into each animal of the series.

In each series the injection was made intradermally into the flank of fully grown, adult animals of either sex. Excepting blood serum, which was used undiluted immediately after formation, each organ extract was prepared by grinding 0·5 g. of freshly removed material with acid-treated silver sand in a small volume of physiological saline. The resultant suspension was centrifuged and the volume of the supernatant fluid increased to 5 c.c. by the addition of saline. The extracts were injected either when they were fresh or after they had been kept in a refrigerator (4° C.) for a period of time varying from 10 to 14 days. Immediately prior to injection a known volume of the extract was mixed with an equal volume of 5 % Trypan blue diluted 1 : 3 with physiological saline and 0·2 c.c. of the mixture injected intradermally into the shaved flank of the animal. In the case of extracts of rabbit choroid plexus additional tests were made using a 1 % double solution of potassium ferrocyanide and iron ammonium citrate instead of Trypan blue.

A suitable control injection of dye plus its own volume of saline was always made in the same animal.

The area of dye was measured in square centimetres 10–30 min. after each injection and then a further measurement taken 24 hr. later. Where Trypan blue was used as the index of spread the dye was clearly visible. In cases where the double solution of potassium ferrocyanide and iron ammonium citrate was injected the blue colouration was less clearly defined. Consequently, these animals were killed 24 hr. after injection and, in order to precipitate Prussian blue, the skin of the flank was placed overnight in a solution containing 10 % formaldehyde and 5 % hydrochloric acid.

Microscopic examination was made of the skin of rabbits injected with extract of rabbit choroid plexus. Small portions of skin from areas injected with extract and with the control solution were excised 24 hr. after injection. When the extract had been injected together with Trypan blue the skin was fixed overnight in Bouin's fluid. When the extract plus the double solution of

potassium ferrocyanide and iron ammonium citrate was injected, the acid formaldehyde solution used to precipitate Prussian blue served also as a fixing agent.

After fixation the portions of skin were dehydrated in a series of suitably graded alcohol solutions, cleared in xylol and embedded in paraffin wax of 56° C. M.P. They were then sectioned in the transverse plane at a thickness of 4 μ and stained in either Weigert's or Ehrlich's haematoxylin and eosin.

(*b*) *Observations on the influence of extracts of choroid plexus on the intradermal spread of dyes*

In all animals of each series, areas injected with dye plus extracts of choroid plexus consistently showed a diffuse appearance, the edges of the coloured area gradually merging into the general skin pigmentation (Pl. I, fig. 1). This was in marked contrast to the control areas and areas where injections had been made with dye plus extracts of either brain or meninges or with blood serum (Pl. I, fig. 2). In these cases the dye was more localized in its distribution and the edges of the injected area were clearly delimited from the surrounding skin.

Furthermore, in the majority of cases the bleb resulting from the injection of dye plus extracts of choroid plexus flattened within 10–30 min. of its formation. The control blebs and those of the extracts of brain, meninges and blood serum became flat only 12–24 hr. after the injection.

The animals of series IV were investigated in order to compare the influence of mammalian testis extract on intradermal spread of dye with that of extract of choroid plexus. In the presence of both organ extracts the dye showed a well-marked diffusion, the edges of the injected areas merging imperceptibly into the surrounding skin colouration. The bleb formed by injection of the testis extract flattened immediately after its formation. A period of 10–30 min. elapsed, however, before the bleb resulting from injection of plexus extract became flat.

Our findings relative to the effect of the different organ extracts on the extent of intradermal spread of dye are summarized in Tables I and II. The average ratio in series I, II and III of spread of extract of choroid plexus to spread of control is diagrammatically represented in Text-fig. 1.

It is seen that:

(1) Extracts of choroid plexus increased the intradermal spread of dye to a far greater extent than did the extracts of meninges, brain and blood serum. Indeed, extracts of brain and blood serum have very little or no effect on intradermal spread, while extract of meninges increased the spread only slightly.

(2) There was a marked diminution in the "spreading" potency of extract of rabbit choroid plexus when injected into guinea-pigs and dogs compared with the potency of the same extract when injected into rabbits. Likewise, extract of

Table I

Series	Species injected	Organ extract	Dye used as index of spread	No. of tests	Ratio of spread of extract to spread of control in individual tests*	Average ratio of spread of extract to spread of control
I	Rabbit	Rabbit choroid plexus (fresh)	Trypan blue	10	9·9 : 1, 7·2 : 1, 10·0 : 1, 5·0 : 1, 5·0 : 1, 8·5 : 1, 5·2 : 1, 8·8 : 1, 9·8 : 1, 10·6 : 1	8·0 : 1
I	Rabbit	Rabbit choroid plexus (stale)	Trypan blue	7	6·0 : 1, 4·9 : 1, 6·0 : 1, 6·0 : 1, 7·0 : 1, 5·0 : 1, 9·2 : 1	6·3 : 1
I	Rabbit	Rabbit choroid plexus (fresh)	Double solution of potassium ferrocyanide and iron ammonium citrate	8	4·5 : 1, 8·5 : 1, 6·1 : 1, 9·1 : 1, 6·6 : 1, 9·7 : 1, 7·2 : 1, 9·7 : 1	7·7 : 1
I	Rabbit	Cow choroid plexus (fresh)	Trypan blue	10	4·7 : 1, 2·2 : 1, 2·0 : 1, 2·3 : 1, 2·3 : 1, 1·5 : 1, 1·5 : 1, 2·0 : 1, 2·6 : 1, 1·5 : 1	2·3 : 1
I	Rabbit	Cow choroid plexus (stale)	Trypan blue	10	4·0 : 1, 2·3 : 1, 2·0 : 1, 2·4 : 1, 1·9 : 1, 2·6 : 1, 1·4 : 1, 1·2 : 1, 2·3 : 1, 2·1 : 1	2·2 : 1
I	Rabbit	Sheep choroid plexus (fresh)	Trypan blue	10	2·6 : 1, 3·0 : 1, 1·9 : 1, 3·2 : 1, 2·0 : 1, 2·4 : 1, 1·9 : 1, 3·3 : 1, 2·7 : 1, 2·5 : 1	2·5 : 1
I	Rabbit	Sheep choroid plexus (stale)	Trypan blue	10	4·8 : 1, 3·4 : 1, 2·0 : 1, 6·8 : 1, 2·3 : 1, 1·7 : 1, 4·0 : 1, 4·5 : 1, 3·9 : 1, 2·8 : 1	3·6 : 1
I	Rabbit	Dog choroid plexus (fresh)	Trypan blue	6	1·1 : 1, 1·8 : 1, 1·6 : 1, 1·9 : 1, 1·6 : 1, 1·6 : 1	1·6 : 1
I	Rabbit	Rabbit meninges (fresh)	Trypan blue	10	1·5 : 1, 2·3 : 1, 1·6 : 1, 1·9 : 1, 1·6 : 1, 1·8 : 1, 3·0 : 1, 1·3 : 1, 1·2 : 1, 1·3 : 1	1·7 : 1
I	Rabbit	Rabbit meninges (stale)	Trypan blue	10	1·2 : 1, 1·8 : 1, 1·2 : 1, 1·4 : 1, 1·4 : 1, 2·3 : 1, 1·4 : 1, 1·5 : 1, 2·8 : 1, 1·3 : 1	1·6 : 1
I	Rabbit	Rabbit brain (fresh)	Trypan blue	10	0·9 : 1, 1·0 : 1, 0·9 : 1, 1·2 : 1, 1·3 : 1, 1·1 : 1, 1·9 : 1, 0·2 : 1, 1·5 : 1, 0·5 : 1	1·1 : 1
I	Rabbit	Rabbit brain (stale)	Trypan blue	10	0·8 : 1, 0·8 : 1, 0·8 : 1, 1·2 : 1, 1·1 : 1, 1·6 : 1, 0·8 : 1, 0·8 : 1, 0·5 : 1, 0·6 : 1	0·9 : 1

* One animal was used for two tests—one test on each flank. The ratios obtained in each animal are bracketed together.

Table I (*continued*)

Series	Species injected	Organ extract	Dye used as index of spread	No. of tests	Ratio of spread of extract to spread of control in individual tests*	Average ratio of spread of extract to spread of control
I	Rabbit	Rabbit blood serum (fresh)	Trypan blue	10	0·6 : 1, 0·7 : 1, 1·1 : 1, 1·3 : 1, 0·7 : 1, 1·2 : 1, 1·1 : 1, 1·4 : 1, 1·3 : 1, 0·9 : 1	1 : 1
II	Guinea-pig	Rabbit choroid plexus (fresh)	Trypan blue	10	2·2 : 1, 1·2 : 1, 1·5 : 1, 2·9 : 1, 1·4 : 1, 3·0 : 1, 5·0 : 1, 5·7 : 1, 5·2 : 1, 4·6 : 1	3·3 : 1
II	Guinea-pig	Dog choroid plexus (fresh)	Trypan blue	10	2·8 : 1, 1·7 : 1, 2·0 : 1, 1·4 : 1, 1·6 : 1, 1·4 : 1, 1·9 : 1, 1·7 : 1, 1·4 : 1, 1·2 : 1	1·7 : 1
III	Dog	Dog choroid plexus (fresh)	Trypan blue	8	11·0 : 1, 11·0 : 1, 8·6 : 1, 8·8 : 1, 2·6 : 1, 3·0 : 1, 2·7 : 1, 4·2 : 1	6·5 : 1
III	Dog	Rabbit choroid plexus (fresh)	Trypan blue	8	2·6 : 1, 2·3 : 1, 1·5 : 1, 1·1 : 1, 2·7 : 1, 1·8 : 1, 3·7 : 1, 1·2 : 1	2·1 : 1
III	Dog	Cow choroid plexus (fresh	Trypan blue	8	2·1 : 1, 2·3 : 1, 2·1 : 1, 1·4 : 1, 1·7 : 1, 2·3 : 1, 1·7 : 1, 1·2 : 1	1·9 : 1
III	Dog	Sheep choroid plexus (fresh	Trypan blue	8	2·0 : 1, 1·8 : 1, 4·0 : 1, 1·3 : 1, 2·1 : 1, 1·4 : 1, 3·2 : 1, 1·3 : 1	2·1 : 1

Table II

Series	Species injected	Organ extract	Dye used as index of spread	No. of tests	Ratio of spread of plexus extract to spread of control in individual tests	Ratio of spread of testis extract to spread of control in individual tests	Ratio of spread of plexus extract to spread of testis extract in individual tests	Average ratio of spread of plexus extract to spread of testis extract
IV	Rabbit	(*a*) Rabbit choroid plexus (fresh)	Trypan blue	6	4 : 1, 14 : 1, 4 : 1, 15 : 1, 13 : 1, 19 : 1	8 : 1, 18 : 1, 11 : 1, 22 : 1, 15 : 1, 31 : 1	1 : 1, 1 : 2, 1 : 1·2, 1 : 2, 1 : 1·4, 1 : 3	1 : 1·7
		(*b*) Rabbit testis (fresh)						

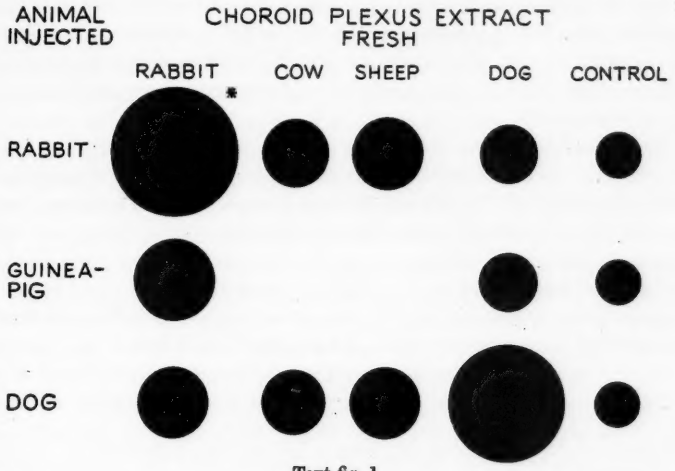
dog choroid plexus was less active when injected into rabbits and guinea-pigs than when injected into dogs (Pl. 1, fig. 3).

(3) Extract of rabbit choroid plexus injected into rabbits was more active than extract of cow or sheep plexus injected into the same animal. Again, extract of dog choroid plexus injected into dogs was more active than extract of cow or sheep plexus injected into dogs.

(4) In the majority of tests, extract of rabbit testis increased the intradermal spread of dye to a greater extent than did extract of rabbit choroid plexus.

(5) The age of the extracts had no effect on their capacity, however slight it be, for facilitating intradermal spread.

We may conclude, therefore, that a factor is present in choroid plexus extracts which possesses the property of altering tissue permeability and thereby



Text-fig. 1.

* This injection was repeated using a 1% double solution of potassium ferrocyanide and iron ammonium citrate as the index of spread. The average ratio of spread of extract to spread of control was approximately the same as in the tests where Trypan blue was used.

increasing the extent of spread of injected materials. This factor appears to be *species specific* in its action since extract of choroid plexus of a particular species is more active when injected intradermally into an animal of the same species, than when injected into an animal of a different species. The fact that the age of the extracts in no way influences their spreading power minimizes the possibility of the factor being a product of autolysis. Finally, although the spreading property of plexus extract is well marked it does not on the whole appear to be as great as that of testis extract.

(*c*) *Examination of skin injected with extracts of choroid plexus*

In section, the skin of areas injected with extract of rabbit plexus and that of the control areas showed a marked difference in macroscopic appearance. In the control areas the skin was of normal thickness and the extent of

the dyes, both Trypan blue and Prussian blue, was easily discerned with the naked eye. In areas injected with dye plus plexus extract, the skin was relatively much thicker and the dye was so diffuse that it could be distinguished only with great difficulty. In the majority of tests made with extract plus the double solution of potassium ferrocyanide and iron ammonium citrate, the usual Prussian blue reaction was obtained in an acid medium. In a few, however, no blue precipitate was formed even after the skin had been immersed in the acid formaldehyde solution for 24 hr. Instead, the injected area in these cases showed a light brown colouration which was coextensive with the thickening of the skin. The significance of this fact is not at present understood, but it is of interest to note that Hoffman and Duran-Reynals (1931), making a similar investigation with mammalian testis extract, apparently encountered no such phenomenon.

Microscopically, the skin of the control areas presented a normal histological picture. In areas injected with dye plus plexus extract marked signs of oedema were observed. The fibre bundles of the dermis were distorted from their normal course and showed enlargement often accompanied by splitting into fibrils. Further, pyknotic nuclei were relatively much more numerous in the dermis of areas injected with extract than in that of control areas.

In areas where injections had been made of extract plus Trypan blue, the dye was washed out during processes of technique and therefore its distribution could not be observed microscopically. In areas injected with extract plus the double solution of potassium ferrocyanide and iron ammonium citrate, the Prussian blue granules had extended more widely through the intercellular spaces of the dermis than in the control areas. Further, the cells of the dermis frequently showed slight invasion by the dye. In the control areas, on the other hand, the dye was strictly confined within the intercellular spaces.

IV. THE ROLE OF THE CHOROID PLEXUS IN THE MECHANISM UNDERLYING THE FIRST EXTRACEREBRAL SPREAD OF FLUIDS INJECTED INTO THE CENTRAL CANAL OF THE EMBRYONIC NERVOUS SYSTEM

(*a*) *Methods of investigation*

To anticipate the extracerebral spread of fluids injected into the central nervous system, chick embryos of accurately determined age were used. The eggs were incubated at a temperature of 38–40° C., care being taken that these limits were not exceeded.

Immediately prior to injection the embryo, together with a portion of the extra-embryonic yolk sac, was removed from the egg and placed in physiological saline warmed to a temperature of 38–40° C. The embryo was exposed by removal of the chorion and amnion and kept in the warm saline throughout the injection. The fluid used for injection was a mixture in equal quantities of a 0·8 % double solution of potassium ferrocyanide and iron ammonium citrate

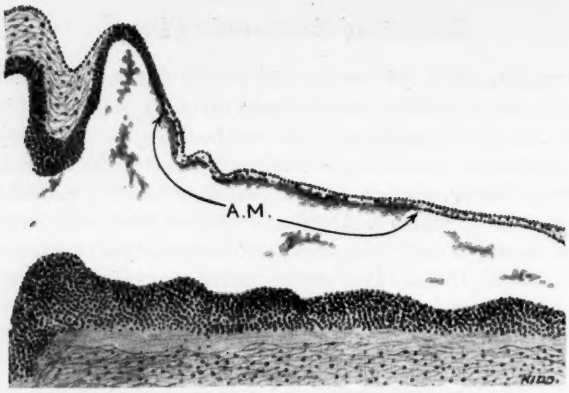
plus either (i) extract of chicken choroid plexus, (ii) extract of cow choroid plexus, or (iii) extract of rabbit testis. Each extract was prepared in the usual manner. A detailed description of the method employed for injecting the embryonic central nervous system has been reported previously (Cohen & Davies, 1937). Briefly, the solution was injected into the caudal end of the central canal of the spinal cord. Any fluid contained within the central nervous system was simultaneously withdrawn from the lateral ventricle of either cerebral hemisphere, thereby avoiding undue increase in intracerebral pressure.

The embryos were injected between the age limits of 4 and $6\frac{1}{2}$ days at intervals of approximately 12 hr. At least two embryos were injected at each stage of development.

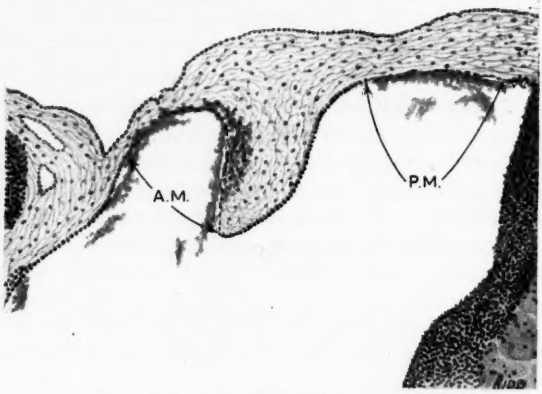
After each injection the embryo, still in the warm saline, was kept alive in an incubator at 38–40° C. for a period of $\frac{1}{2}$–1 hr. to allow of any possible spread of the injected solution. The embryonic heart was easily observed through the transparent integument and served as an index of continued circulation. The embryos were then fixed overnight in a solution containing 10% formaldehyde and 1% hydrochloric acid. At the same time the acid in the fixing agent secured precipitation *in situ* of Prussian blue, and thus revealed the path taken by the injected solution. After fixation was complete the embryos were dehydrated, cleared and embedded in the customary manner. They were then serially sectioned in the longitudinal plane at a thickness of 4 μ and stained in either Weigert's or Ehrlich's haematoxylin and eosin.

(*b*) *Observations on the influence of extracts of choroid plexus and rabbit testis on the permeability of specialized areas in the roof of the embryonic fourth ventricle*

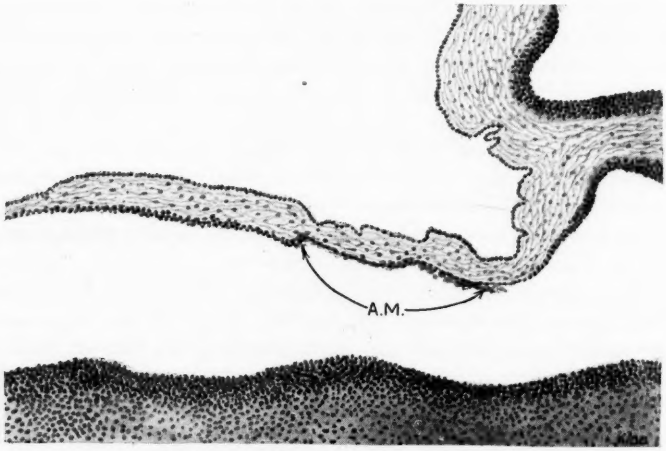
A detailed account of the development of the roof of the fourth ventricle and choroid plexuses in the chick has already been published (Cohen & Davies, 1937) so that a brief statement will suffice here. At certain stages in development two membranous areas, one anterior and the other posterior, are present in the roof of the fourth ventricle. The anterior area is first differentiated in embryos of $3\frac{1}{2}$ days. It then increases rapidly in extent till at $4\frac{1}{2}$ days it occupies the major portion of the anterior region of the myelencephalic roof. At 5 days this area shows a slight reduction in size and thereafter gradually diminishes till at 9 days it is vestigial. The posterior area is fully differentiated in embryos of $6\frac{1}{2}$–$6\frac{3}{4}$ days' incubation. It increases rapidly during successive stages till at 9 days it occupies practically the entire medullary roof posterior to the choroid plexus. The first extracerebral spread of fluid injected into the central nervous system is seen in embryos of 7 days. At this stage and at $7\frac{1}{2}$ days the anterior membranous area alone serves as the site of fluid escape. At 8, $8\frac{1}{2}$ and 9 days, extraventricular escape of fluid occurs through the posterior membranous area only. The first choroid plexuses to develop are those of the lateral and third ventricles. Their complete differentiation at



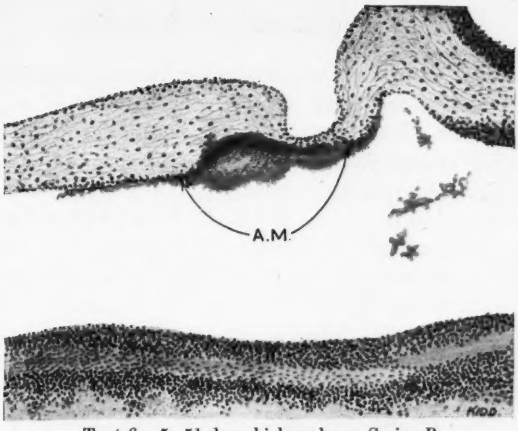
Text-fig. 2. 4½-day chick embryo, Series *A*.



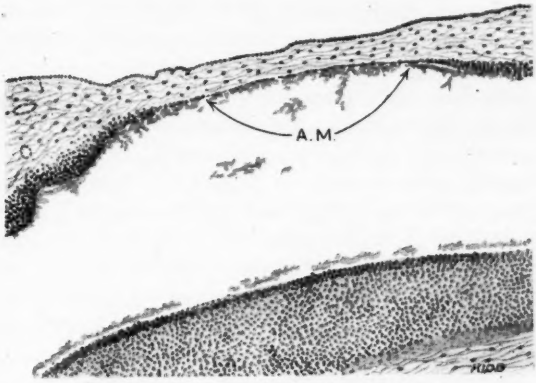
Text-fig. 3. 5-day chick embryo, Series *A*.



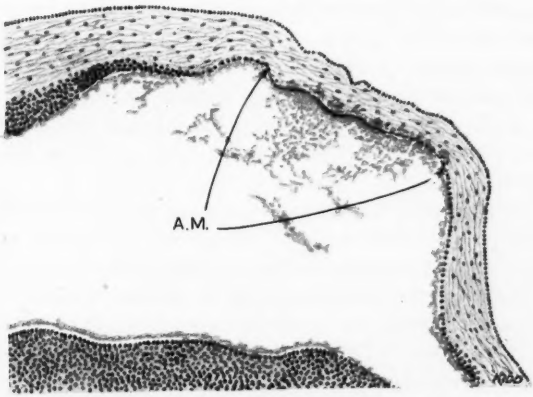
Text-fig. 4. 5-day chick embryo, Series *B*.



Text-fig. 5. 5½-day chick embryo, Series *B*.



Text-fig. 6. 4½-day chick embryo, Series *C*.



Text-fig. 7. 5½-day chick embryo, Series *C*.

7 days is coincident with the first extracerebral spread of fluid. The choroid plexus of the fourth ventricle is fully developed between 8½ and 9 days, and this is correlated with a further marked escape of fluid.

The presence in mammalian testis of a factor increasing tissue permeability has been established by several investigators. Our own research has demonstrated the presence in the choroid plexus of a factor with a similar property. To compare the influence of both plexus and testis extracts on the permeability of the specialized areas in the myelencephalic roof, three series of chick embryos, series A, B and C were investigated. In series A the embryonic central nervous system was injected with a double solution of potassium ferrocyanide and iron ammonium citrate plus extract of chicken choroid plexus. In embryos of series B the double solution, plus extract of cow choroid plexus was injected, while in the embryos of series C, extract of rabbit testis was used instead of plexus extract.

Our observations on all three series are briefly recorded below.

Series A. (Injected with a double solution of potassium ferrocyanide and iron ammonium citrate *plus extract of chicken choroid plexus.*) In embryos of 4 and 4½ days (see Text-fig. 2) a heavy condensation of dye, intermingled with coagulum, was seen adhering to the ventricular surface of the membranous area in the anterior portion of the myelencephalic roof. The cells of this area were heavily impregnated with the dye and a slight infiltration of blue precipitate had occurred here and there through the area into the overlying mesenchyme. In embryos of 5, 6 and 6½ days' incubation (see Text-fig. 3) there was a more marked extraventricular spread of injected fluid through the anterior membranous area. A dense accumulation of blue granules adhered to the ventricular surface of the slightly differentiated posterior area but there was no evidence of fluid escape from this region.

Series B. (Injected with a double solution of potassium ferrocyanide and iron ammonium citrate *plus extract of cow choroid plexus.*) The embryos of this series were injected only between the age limits of 4 and 5½ days.

In embryos of 4, 4½ and 5 days (see Text-fig. 4) a dense precipitate of Prussian blue granules was seen below the anterior membranous area, but there was no evidence of fluid escape from this region. In embryos of 5½ days (see Text-fig. 5) a slight extracerebral spread of the injected solution had occurred, some of the dye having filtered through the membranous area into the overlying mesenchyme.

Series C. (Injected with a double solution of potassium ferrocyanide and iron ammonium citrate *plus extract of rabbit testis.*) In embryos of 4½ and 5 days' incubation (see Text-fig. 6) an accumulation of Prussian blue granules was seen below the anterior membranous area. At these stages no extracerebral spread of fluid had occurred, the injected solution being strictly confined within the spaces of the central nervous system. In embryos of 5½ and 6 days (see Text-fig. 7) there was still no evidence of marked extraventricular escape, merely a slight infiltration of the dye through the membranous area into the

overlying mesenchyme. A heavy condensation of blue precipitate adhered to the ventricular surface of the slightly differentiated posterior area but no fluid escape had occurred from this region.

Thus, extracts of cow choroid plexus and of rabbit testis increase the permeability of the membranous areas but slightly and only at a comparatively late stage in development (5½ days). Extract of chicken choroid plexus, on the other hand, renders these areas slightly permeable to dye at an early stage (4 days) and more markedly so as development proceeds (5–6½ days).

V. GENERAL SUMMARY AND DISCUSSION

The results of the present investigation indicate that extract of choroid plexus contains a factor having the power of increasing tissue permeability. Extract of rabbit meninges possesses a similar property but to a lesser degree, while extract of rabbit brain and blood serum have no such effect on tissue permeability. We found that dye injected intradermally with rabbit brain extract remains localized in its distribution and that the average ratio of spread of extract to spread of control was either 1·1 : 1 (fresh extract) or 0·9 : 1 (stale extract). Claude & Duran-Reynals (1934), who made a similar investigation, found that of ten tests made with extract of rabbit brain nine yielded a positive result with increased diffusion of the dye. Further, the average ratio of spread of the extract to spread of the control is quoted as 1·9 : 1. These authors state that their tests involved the extract of an entire organ. We suggest, therefore, that in their preparation of brain extract the choroid plexus was involved and that this organ, not the brain tissue, was responsible for the slight increase in the spread of the dye.

Extracts of choroid plexus and of mammalian testis are similar in that both facilitate the intradermal spread of injected material. Further, in the presence of choroid plexus extract the fibre bundles of the dermis become enlarged, distorted from their normal course, and frequently show splitting into fibrils. Thus, the histological picture resulting from injection of plexus extract resembles that produced by injection of testis extract, described by McClean (1931). Our findings in animals of series 4 (see Table II) suggest that extract of choroid plexus is less potent than that of testis. Only in one test was the spread of both organ extracts approximately the same, while in one case the spread of the testis extract was three times as great as that of plexus extract. Moreover, the blebs resulting from injection of testis extract flattened immediately after their formation, those of plexus extract only within 10–30 min. of the injection.

Unlike extract of mammalian testis, plexus extract tends to show species specificity in its action. The ratio of spread of extract to spread of control is greater in rabbits injected with rabbit plexus extract than in guinea-pigs and dogs injected with the same extract. Again, the ratio is greater in dogs injected with extract of dog plexus than in rabbits and guinea-pigs similarly injected. Additional evidence of the species specific action of choroid plexus extract is

obtained by comparing the behaviour of extract of rabbit plexus when injected into rabbits with that of cow and sheep plexuses also injected into rabbits. In the former case the average ratio of spread of extract to spread of control is considerably greater than in the latter. Similarly, the ratio is greater in dogs injected with extract of dog choroid plexus than in dogs injected with extracts of cow or sheep plexus.

Previous investigations (Cohen & Davies, 1937, 1938) have established a correlation between the first extraneural spread of fluids injected into the embryonic central nervous system and the development of the choroid plexuses. The present investigation throws considerable light on the precise nature of this correlation.

Observations on the chick embryos of series A show that when extract of chicken choroid plexus is injected into the central nervous system, extraventricular escape of fluid occurs at stages prior to the development of the embryonic plexus. In view of the fact already established that choroid plexus extract influences tissue permeability, the inference is that the plexus extract increases the permeability of the membranous areas in the myelencephalic roof which then permit of fluid escape. We suggest, therefore, that the primary function of the choroid plexus in the earlier stages of its development is the elaboration of a factor which renders permeable the membranous areas and so initiates the first extracerebral escape of fluids from the central nervous system.

The embryos of series B show that extract of cow choroid plexus increases the permeability of the membranous areas only slightly. Embryos of series C show that extract of rabbit testis has a similarly slight effect—an observation which is surprising in view of the very marked capacity this extract has of increasing tissue permeability. Our evidence suggests that the permeability of the membranous areas in a particular embryonic type is influenced by a factor elaborated only by the choroid plexuses of animals of the same species.

VI. CONCLUSIONS

1. Extract of choroid plexus contains a factor which has the power of increasing tissue permeability. A similar property is shown by extract of meninges but in much less degree, while it is entirely lacking in extract of brain tissue.

2. The factor present in choroid plexus extract is *species specific* in its influence on tissue permeability.

3. Experimental evidence is recorded suggesting that the factor elaborated by the choroid plexus plays an important part in the mechanism underlying the first extraventricular escape of fluid from the embryonic central nervous system, and it is suggested that it exerts its influence by increasing the permeability of the membranous areas in the myelencephalic roof, thus permitting the extraneural escape of intraventricular fluid.

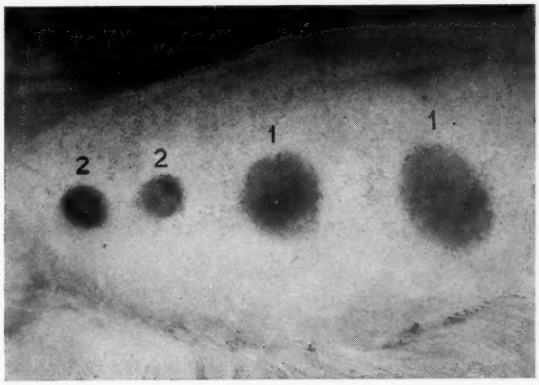


Fig. 1.

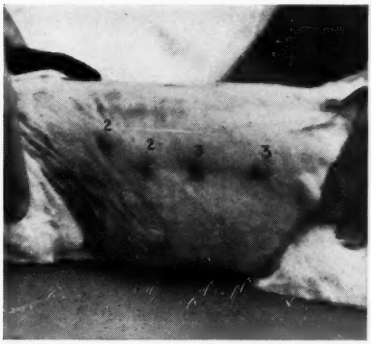


Fig. 2.

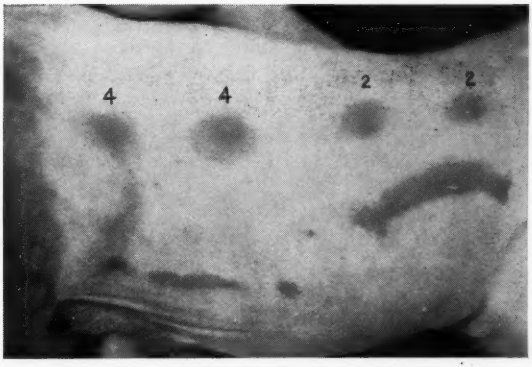


Fig. 3.

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EXPLANATION OF PLATE I

Fig. 1. Flank of rabbit showing anteriorly areas injected intradermally with control solution (marked 2) and posteriorly areas similarly injected with extract of rabbit choroid plexus plus Trypan blue (marked 1).

Fig. 2. Flank of rabbit showing anteriorly areas injected intradermally with control solution (marked 2) and posteriorly areas similarly injected with extract of rabbit brain plus Trypan blue (marked 3).

Fig. 3. Flank of rabbit showing anteriorly areas injected intradermally with control solution (marked 2) and posteriorly areas similarly injected with extract of dog choroid plexus plus Trypan blue (marked 4).

ABBREVIATIONS

A.M. Anterior membranous area. *P.M.* Posterior membranous area.

PARTIAL DEGENERATION OF THE NERVE SUPPLY OF THE ADRENAL. A STUDY IN AUTONOMIC INNERVATION[1]

By J. Z. YOUNG

Department of Zoology and Comparative Anatomy, Oxford

It is now generally held that in vertebrate striped muscle each motor nerve fibre sends branches to a definite set of muscle fibres. The operations of the muscle as a whole are then controlled by the calling into action of the muscle fibres in varying numbers and to varying extents by the central activation of their motoneurons. For tissues other than striped muscle there is no general agreement as to the arrangement of the nerve supply. It has been thought by some histologists (see Stöhr, 1935) that the peripheral fibres in smooth muscle and glandular tissue constitute a continuous network, and the view has been developed, on physiological as well as anatomical grounds, that no discrete functional units can be recognized in such tissues.

The problem is clearly of the greatest importance for the analysis of the mechanism by which graded action of these tissues is produced, and it seemed that a contribution to its solution might be made by study of the adrenal glands. For if the hypothesis of Elliott (1913) that the innervation of the chromaffin tissue is preganglionic be correct, then it should be possible to cut some only of the roots supplying these fibres, and to see whether the subsequent degeneration in the adrenal medulla is generalized or localized.

A decision on the question of whether the innervation of this tissue is really preganglionic was made especially desirable following the demonstration by Feldberg *et al.* (1934) that acetylcholine is liberated in the adrenal glands during stimulation of the splanchnic nerves. In the dogfish there are sympathetic cells so close to the suprarenal bodies that their processes can be followed all the way to their termination around the chromaffin cells (Young, 1933). In this case, therefore, some of the chromaffin cells are innervated by post-ganglionic fibres. The critical experiment which would settle the question is to look, in a mammal, for degeneration of the finest fibres in the adrenal medulla itself after section of spinal roots. At the time this work was begun no such experiments had been reported, but in the meanwhile papers have appeared by Hollinshead (1936) and Swinyard (1937) dealing with the question. Their

[1] The experimental portion of this work was done in the Department of Anatomy of the University of Chicago during tenure of a Fellowship of the Rockefeller Foundation. It is a pleasure to thank the members of that Department, and especially Prof. G. W. Bartelmez, for their assistance. I am also greatly indebted to Mr E. J. Largent for the great skill with which he performed the histological part of the work. Prof. E. S. Goodrich, F.R.S., has kindly read the MS. of the paper.

experiments agree with my own in showing that in the cat the innervation of the adrenal glands is preganglionic, as Elliott supposed. However, a number of further important questions about the innervation are not fully dealt with by these authors. (1) Are *all* of the fibres preganglionic? (2) Over what spinal roots do they emerge? (3) In which of the splanchnic nerves do they run? (4) Does section of some of the fibres to the adrenal lead to the production of *localized* areas of degeneration?

METHODS

The possibility of studying localized degeneration within the adrenal medulla depends on obtaining preparations in which the nerve fibres are uniformly stained, so that one can be certain that any area showing no fibres is really free of them. Complete success in this respect was obtained with the copper-protargol, silver-on-the-slide, technique of Bodian (1936). This method gives a brilliant and uniform stain of the fibres in the adrenal medulla (except the very finest, which are only sometimes stained). It works after fixation in various ways, and for most of the present series Carnoy's alcohol-chloroform-acetic fluid was used. The glands were embedded in paraffin and sectioned in the horizontal plane at 15 μ. Sample series were then mounted, taking one section out of every five or ten, and stained with Bodian's method.

In its original form the method stains the nerve fibres satisfactorily, but also gives a purple colour to the glandular tissue. This makes it difficult to pick out the nerve fibres, especially at low magnifications. By the use of a modification discovered by Mr E. J. Largent it was possible to suppress this staining of the background. The slides are removed from the protargol to distilled water for 10 min., then placed in 1 % oxalic acid for about 2 min. and then again in distilled water for 5 min. before transferring to the reducer. The degree of suppression of the stain depends on the length of stay in oxalic acid, and if this is further prolonged the nerve fibres themselves will not be stained.

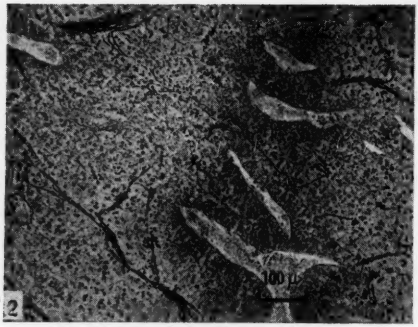
Operations were all performed on cats under nembutal anaesthesia. After laminectomy the dorsal and ventral roots were cut together, close to the dura. Section of the splanchnic nerves was performed after laparotomy.

The times of survival allowed for degeneration were between 5 and 8 days. The tissues were removed at biopsy under nembutal, and all pieces fixed immediately on removal. The operations were performed on one side only, the opposite adrenal being always fixed, embedded and stained as a control. Careful verification of the lesion was made in all cases, the nerves being sectioned serially or teased and stained with osmium tetroxide when necessary.

SECTION OF SPINAL ROOTS

It was found that after section of certain spinal roots degeneration occurred in the ultimate nerve fibres of the adrenal medulla. In the earliest experiments in which the hinder thoracic roots were cut it at once became evident that only

part of the gland had become denervated. Fig. 1 shows a low power view of the medulla in which degeneration of the fibres of the anterior end[1] of the gland had occurred as a result of section, 9 days previously, of D 2 to D 11. It is very difficult to show the finer nerve fibres in such a low power photograph, but Figs. 2 and 3 show small portions of the normal and denervated portions of the gland at higher magnification.



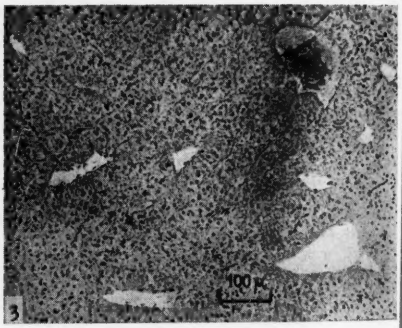


Fig. 1. Cat BN. Spinal roots D 2–11 cut 9 days previously. The head end lies to the right of the figure and is denervated. All the figures are untouched photographs of sagittal sections of the adrenal medulla, prepared with the modified Bodian method described in the text.

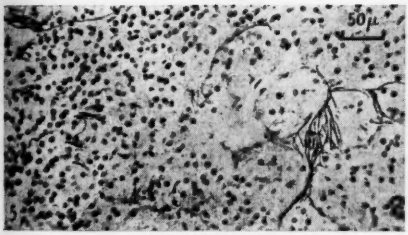
Fig. 2. Cat BN. Portion of the innervated area seen to the left of Fig. 1.

Fig. 3. Cat BN. Portion of the denervated area seen to the right of Fig. 1. Notice one small intact piece to be seen on the right of the figure.

A very interesting question is whether the lines of demarcation between intact and denervated areas is a sharp one. The question is not easy to decide since it is difficult to obtain any quantitative estimate of the degree of innervation in any given microscopic field. At the boundary of innervated and

[1] It is not easy to say exactly which is the "front" end of the adrenal on account of the oblique position of the gland. From the results here described it appears that the morphological axis corresponds with the long axis, although this does not lie exactly along the main antero-posterior line of the body.

denervated areas there sometimes appears to be a region in which fewer bundles are seen than in the normal parts of the gland. These bundles however are not the finest fibres, and it is probable that the plexus around any one chromaffin cell (Willard, 1936) degenerates or remains intact as a whole. The preparations, however, are not adequate to reach a definite decision on this important point. It is not therefore possible to decide whether any one chromaffin cell can receive stimuli from the processes of more than one nerve cell.



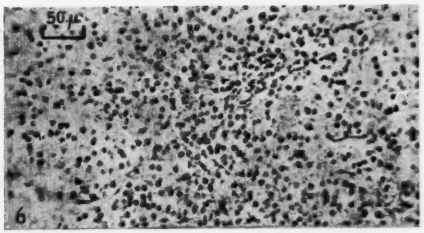


Fig. 4. Cat BY. Spinal roots D 7–17 cut 11 days previously. The anterior end lies to the left of the figure and still contains bundles of nerve fibres.
Fig. 5. Cat BY. Portion of the innervated area seen on the left of Fig. 4.
Fig. 6. Cat BY. Portion of the denervated area seen on the right of Fig. 4.

After a few preliminary observations, eight critical experiments were made, in which various spinal roots were cut. The results of these operations are summarized in Fig. 7. In each case a section showing the greatest extent of the medulla on the operated side was chosen, and projected at a magnification of twenty times. The outline of the whole gland and the boundary of the denervated area was then drawn as carefully as possible, and the resulting pictures reduced to the magnification shown in the figure. This procedure gives a good idea of the proportion of the gland which has been denervated, but, naturally, somewhat different proportions of innervated and denervated tissue will appear in other sections.

It will be seen that the highest root whose section produced degeneration was D 7 (animal CK). In the cat BY, although D 7 was cut, a considerable area at the front end of the gland still contained fibres, so that the level at which adrenal fibres emerge is higher in BY than in CK. In two cases (BO and BN) section of roots down to D 11 produced denervation of about half of the gland. In BQ, with roots sectioned down to D 15 (L 2), a small area at the hind end still contained fibres, but in CL, with D 3–16 cut, total denervation was produced (Plate I, figs. 2, 4). The only nerve fibres remaining in this medulla

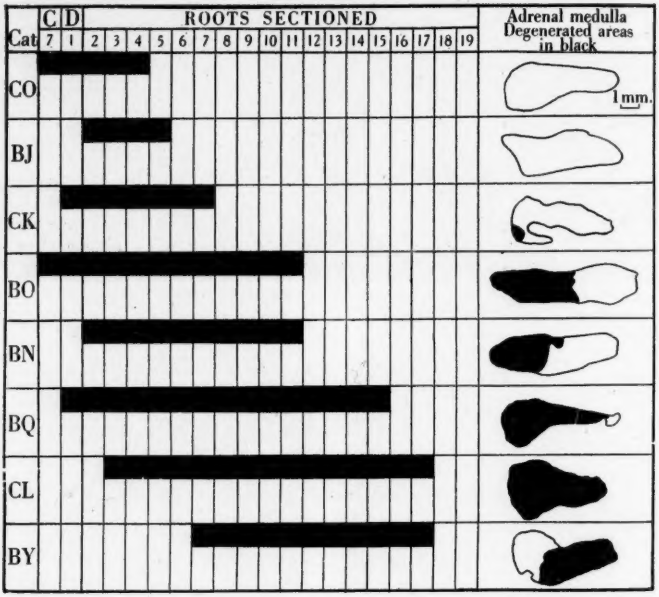


Fig. 7. Summary of degeneration in the adrenal medulla following section of spinal dorsa and ventral roots. The black rectangle shows the nerves cut at operation. The figures to the right show in black the area of denervation found in the largest sagittal section of the medulla on the operated side.

are a very few short pieces, most of which are abnormally thick and are probably cut but unresorbed fibres, and a number of fibres running with the blood vessels. This makes a striking contrast with the normal dense plexus.

The finding that most of the fibres for the adrenal medulla arise from the hinder thoracic roots agrees with the observations of Swinyard (1937). The maximum of degeneration which he observed, however, was "50–70 % of the nerve bundles which entered the gland", this being in animals in which roots D 6 to L 2 were cut. According to my observations such an operation would produce degeneration of all of the fibres around the chromaffin cells, except perhaps in a small area at the hind end of the gland. The discrepancy is

certainly partly accounted for by the fact that Swinyard counted "nerve bundles", many of which he agrees contain postganglionic fibres for the blood vessels. From the present series, however, using the criterion not of bundles but of the presence or absence of fibres among the chromaffin cells, there is no escape from the conclusion that all, or very nearly all of the secreto-motor fibres are preganglionic and uncrossed. The evidence of the sections, such as that shown (Plate I, fig. 2) is quite unambiguous on this point, only a very few scattered fibres are present in the glands on the operated side, although they are abundant and uniformly distributed throughout the control glands. The Bodian method of staining gives such uniform and certain results that it is possible to rely on the significance of the absence of fibres, about which one would be doubtful after impregnation in the block.

As has been stated above, the very few scattered fibres which remain (Figs. 3, 6) are probably pieces of cut preganglionics not yet completely degenerated. If any of them are intact fibres they might be considered to be postganglionics or crossed preganglionics. But in any case they are extremely scarce, in most sections none of them can be found at all. From this experiment, therefore, it can be concluded that *there is no significant number either of postganglionic fibres or of crossed preganglionics innervating the chromaffin tissue of the medulla of the cat.* I have reached this conclusion in spite of the belief, based on my observations on the dogfish, that postganglionic innervation of this tissue does occur. It is, indeed, possible, in those mammals in which sympathetic cells are more abundant in and around the medulla than they are in the cat, that some postganglionic fibres run from these cells to the chromaffin tissue.

The question as to the segments of origin of the preganglionic fibres can also now be answered. They all emerge between D 6 and D 16 (L 3), with possible variations a segment up or down in some cases.

No attempt was made to study systematically the time needed for degeneration. In one animal with roots D 1–18 cut, which died 4 days after operation, the fibres in the medulla were intact, though they were somewhat thick and stained abnormally darkly. Seven days after operation degeneration appears to be nearly complete, no lumps or detritus being visible except the very few short thick pieces mentioned above.

SECTION OF SPLANCHNIC NERVES

The twelve critical experiments made in this series are summarized in Fig. 8. In the cat BZ, and in one other not shown in the figure, the nerves to the adrenal were cut as completely as possible, by clearing all around the gland and leaving only the blood vessels intact. This operation produced total denervation, and agrees with the other cats in which this was achieved in showing that neither the small groups of sympathetic cells beneath the adrenal capsule, nor those in the medulla (rare or absent in the cat) contribute significantly to the innervation.

Animals BM and CQ agree in showing that section of the great splanchnic nerve results in denervation of about half of the gland. This does not agree with the conclusion of Hollinshead that the great splanchnic carries very few of the

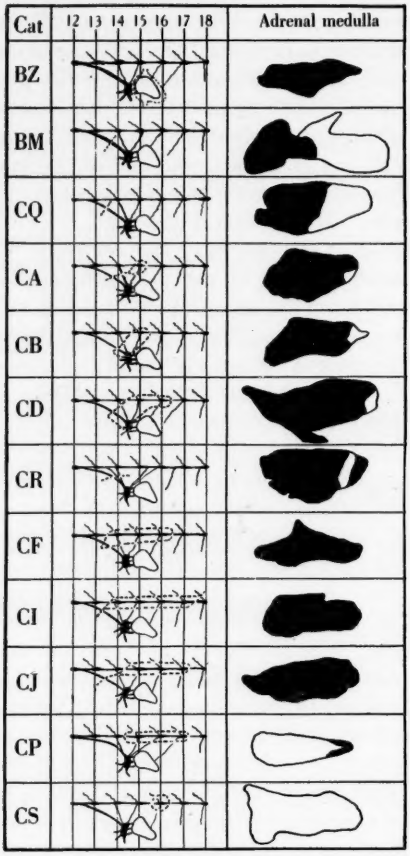


Fig. 8. Degeneration in the adrenal medulla following section of various parts of the splanchnic nerve complex. The arrangement of these nerves is not quite the same in all cats, but has been schematized in the diagrams on the left of the figure. The ruled vertical lines show the spinal nerves. The sympathetic ganglia are shown as lying on the spinal nerves to which they send transverse (post-ganglionic) rami. They receive preganglionic fibres from more anterior spinal nerves in the oblique rami, which are indicated in the figure. The dotted lines show the extent of the lesion at operation. The black areas on the figures to the right show the denervated regions which appear in the largest sections of the medulla on the operated side.

fibres. However, Hoshi (1927) on histological grounds, and many workers on the stimulation of the output of adrenalin, have concluded that many fibres for the medulla are carried in this nerve (see Stewart and Rogoff, 1916).

Cats CA, CB, CD and CR show that most of the fibres which do not run in the greater splanchnics pass through the second or lesser splanchnic nerve, or from nerves arising from or near to the sympathetic ganglion which gives transverse (grey) rami to D 14 and 15. As suggested by the experiments in which spinal roots were sectioned some fibres also pass in roots lower than D 15, and total denervation can only be achieved by cutting the branches emerging from these lower sympathetic ganglia, as has been done in cats CF, CI and CJ. This contribution from lower segments is small, however, as shown by CP, showing a very small denervated area, and CS with none at all.

For complete denervation of the adrenal gland in the abdomen, therefore, a safe procedure, as found also by Hollinshead (1936) and Hollinshead & Finkelstein (1937), is to cut the great splanchnic and to remove a section of the sympathetic chain from the diaphragm back to the ganglion which gives a transverse ramus to D 17 (L 4). It should be noted that all of the present series of operations were done on the left side; the conditions on the right may be slightly different.

Neither in these experiments nor in those in which spinal roots were cut was any evidence of crossed degeneration seen (p. 545), although sections of both adrenals were prepared in every case. This agrees with the results of Hoshi (1927) (using rabbits), but Botar & O'Shaughnessy (1936) imply that section of the left splanchnics causes equal degeneration in both adrenals. They do not say whether the "diminution des fibres nerveuses" which they observed involved total denervation of whole areas or what method they employed for assessing the number of fibres present.

DISCUSSION

The results may be briefly discussed in their bearing on the four questions formulated on p. 541.

(1) In the cat the innervation of the medulla is completely, or very nearly completely, preganglionic. It remains to be seen whether in those mammals which have more numerous intramedullary sympathetic cells these send processes to the chromaffin tissue.

(2) One of the most surprising results of the investigation is the great length of the series of roots which send fibres to the adrenal medulla, extending at least from D 6 to 16. Plurisegmental innervation might be expected from the phylogenetic and ontogenetic history of the chromaffin tissue, but it is interesting to find it so well marked. There is, of course, no certainty that the cell bodies lie in the segments by which the fibres leave the roots. However, there are indications that even reflex excitation of the secretion of adrenalin can be elicited from the lower part of the cord. For instance, Brooks (1933) found increased secretion of adrenalin after stimulation of the sciatics even after the cord had been severed at D 10.

On the other hand, Stewart & Rogoff (1917) found that the "spontaneous" secretion of adrenalin in the resting cat is controlled by cells lying in the cervical

and upper thoracic region, extending back not farther than D 4. This is higher than the level at which fibres begin to leave for the adrenal medulla. We must therefore conclude that this set of cells, which regulates the basic level of adrenalin secretion, controls the activity of the adrenal motoneurons themselves, which lie lower, and probably segmentally, in the cord, and can also be reflexly affected from both higher and lower levels, as in the experiments performed by Brooks.

(3) The distribution of the adrenal fibres in the various splanchnic nerves has already been discussed. Total denervation can only be achieved by cutting all of the splanchnic nerves. Since at least half of the fibres to the adrenal run in the lower branches it is possible that section of these may be of clinical benefit if the claims advanced for the advantages of adrenal denervation can be substantiated. As Rogoff (1936) has pointed out, the danger of the operation as usually performed close to the gland is that damage to the blood supply may result in degeneration of the cortical tissue. Reduction of adrenal secretion could be produced without this danger by section of the lower splanchnic nerves or removal of a section of the sympathetic chain, though the possibility of regeneration cannot be excluded (see Hollinshead & Finkelstein, 1937).

(4) Section of some only of the fibres reaching the adrenal results in the production of local areas of denervation. This result is implied in the observations of Hoshi (1927) and Hollinshead (1936) though they do not discuss it explicitly. The limitation of degeneration to certain areas strongly suggests that each nerve fibre innervates a definite group of chromaffin cells. This implies that the adrenal medulla is divided into a number of functional units, which can be separately recruited by the activation of their motoneurons, just as can the units of a striped muscle.

This quantal arrangement is opposed to that suggested by Stöhr (1935, etc.), who appears to believe that all the processes of the sympathetic cells reaching a tissue constitute a closed network. From what we know of nervous conduction, any one impulse in such a system would presumably spread over the entire network. There is no reason why degeneration should stop in such a network at certain points, or indeed why any degeneration should occur at all so long as connexion with one cell body remains. Speidel (1933) has confirmed the old and general opinion that any connexion with a cell body is sufficient to maintain the integrity of a nerve fibre. The type of plexus suggested by Stöhr does not, therefore, occur in the adrenal medulla.

The only case in which local degeneration of the nerves to a gland or smooth muscle has been studied is that of Lawrentjew & Borowskaja (1936). They found that after section of some of the branches emerging from the superior cervical ganglion some only of the post-ganglionic nerve fibres in the nictitating membrane degenerate. The experiments of Eccles (1936) further indicate that in this same muscle gradation of response is produced by quantal recruitment, as in a striped muscle.

We still require much further information on many points, particularly as to whether any one smooth muscle fibre or gland cell receives impulses from more than one nerve cell. The evidence available strongly suggests that there is no common anastomosing network of either pre- or post-ganglionic fibres, and that the autonomic nervous system is divided into functional units of the same general type as those of the somatic system.

SUMMARY

1. Section of spinal roots in the cat is followed by disappearance of the nerve fibres which innervate the chromaffin cells of the adrenal medulla of the same side. This confirms the findings of Elliott, Hollinshead and Swinyard that the innervation of the chromaffin tissue is preganglionic.

2. The segments over which the adrenal fibres leave the cord are D 6–D 16 (L 3), with possibly a few fibres from higher and lower segments in some cases. By section of this whole series of roots on one side total denervation of the ipsilateral medulla can be achieved. No evidence of contralateral innervation was found.

3. Section of some only of these roots results in the production of local areas of degeneration in the medulla. Segmental relations are preserved, the front end of the gland receiving fibres from the more anterior roots.

4. The separate fibres do not constitute an anastomosing plexus, but each innervates a defined area of the gland.

5. Section of the great splanchnic nerve produces denervation of the anterior half of the medulla. The lesser splanchnics contain most of the rest of the fibres, but a few run through lower sympathetic ganglia. Total denervation of the gland can therefore be assured by cutting the great splanchnics and removing the sympathetic chain from the diaphragm to the ganglion which gives a transverse ramus to D 17 (L 4).

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EXPLANATION OF PLATE I

Fig. 1. Cat CL. Left adrenal medulla. Intact.
Fig. 2. Cat CL. Right adrenal medulla. On this side spinal roots D 3–16 cut 10 days previously. Medulla totally denervated.
Fig. 3. Cat CL. Portion of normal gland shown in Fig. 1.
Fig. 4. Cat CL. Portion of denervated gland shown in Fig. 2.

Fig. 1.

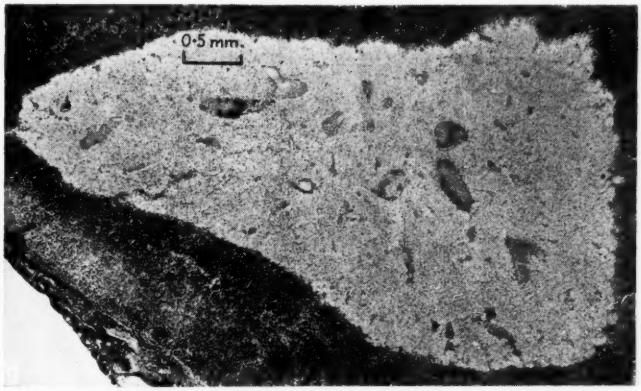


Fig. 2.

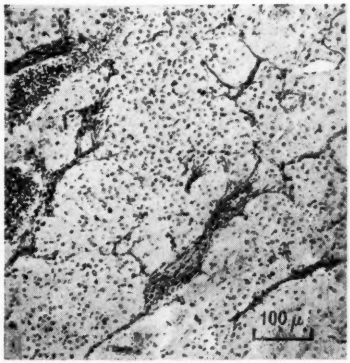


Fig. 3.

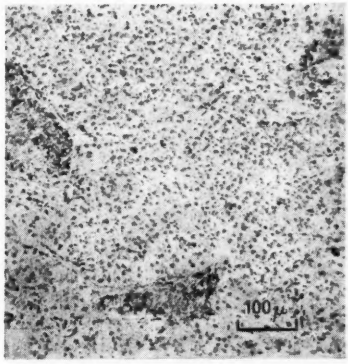


Fig. 4.

UNIV. OF MICH.

AN EXPERIMENTAL INVESTIGATION OF THE NERVE SUPPLY OF THE ADRENAL MEDULLA OF THE CAT

By W. d'A. MAYCOCK and T. STEWART HESLOP[1]

INTRODUCTION

The adrenal medulla in the cat receives its main nerve supply from the greater and lesser splanchnic nerves. Fibres from the lumbar ganglia of the abdominal sympathetic chains also run directly, or through the coeliac ganglia, to the adrenal glands (Elliott, 1913). Apart from a statement by Cannon *et al.* (1926) that stimulation of branches from the lumbar ganglia causes the liberation of adrenaline, no experiments have, to our knowledge, been reported in which the distribution of these nerves or the direct effect of stimulation of them on the secretion of adrenaline has been studied. Botar & O'Shaughnessy (1937), from the histological examination of the adrenal glands of monkeys, cats and dogs after section and removal of various nerves, concluded that the nerve supply reached the adrenal medulla exclusively through the greater and lesser splanchnic nerves and that the supply was bilaterally distributed.

Physiological experiments have been performed on cats to investigate the relative functional importance and distribution of those branches of the sympathetic nervous system supplying the adrenal medulla, and to establish the presence or absence of a crossed nerve supply. The liberation of adrenaline, as shown by the retraction of the denervated nictitating membrane, has been taken as the index of activity of the nerves investigated, and therefore the results reported here do not necessarily apply to all the nerves demonstrable in histological preparations, some of which are probably vasomotor in function.

METHODS

Cats weighing 2·5–3 kg. were used. Seven to ten days before the experiment, the right superior cervical sympathetic ganglion was removed aseptically under ether anaesthesia to sensitize the corresponding nictitating membrane to small quantities of adrenaline. In most cases, for the final experiment, the cat was anaesthetized with ether, and, after the insertion of a tracheal cannula, the left carotid artery was tied and the right clipped with a bull-dog, which was later removed to restore the circulation to the nictitating membrane. The spinal cord was then cut at the level of the second cervical vertebra, and the brain was destroyed by passing a rod through the foramen magnum. Ether was then withdrawn and artificial respiration applied. In some experiments

[1] Leverhulme Scholars of the Royal College of Surgeons of England.

the cats were anaesthetized with "Dial" (Ciba), 1 c.c./kg. body weight intraperitoneally. After an abdominal evisceration including the kidneys, the splanchnic nerves and the lumbar sympathetic chains and their branches were dissected. The chains were lifted free from the psoas muscles, and branches from the ganglia, which entered these muscles, were cut. The chains were sectioned above the first lumbar ganglion and between the third and fourth lumbar ganglia. In such a preparation, stimulation of the splanchnic nerves or branches of the lumbar sympathetic chain can cause retraction of the nictitating membrane only by the liberation of adrenaline from the adrenal glands. The nerves were stimulated through shielded platinum or silver wire electrodes by condenser discharges delivered at a frequency of 20–50 per sec. by a gas discharge tube. The splanchnic nerves were crushed or tied proximal to the site of the electrodes. The blood pressure in the left carotid artery was recorded with a mercury manometer. The nictitating membrane was attached to a light aluminium lever, weighted to give a tension of 6–8 g. and magnifying five times, which recorded its retraction on the smoked drum. The sensitiveness of the membrane was tested by the intravenous injection of adrenaline (2, 1, 0·5 and 0·25 μg.) at the beginning and end of each experiment: usually 0·25 μg. adrenaline produced an easily measurable retraction. In most instances the sensitiveness decreased slightly during the experiment. In a few the decrease was great; in such experiments the results obtained in the early part of the experiment only have been used.

Eserine, 0·2 mg./kg. body weight, was injected in some experiments to increase the sensitiveness of the adrenal gland to nerve stimulation (Tschebok-saroff, 1910; Feldberg *et al.* 1934). The degree of sensitization varied from experiment to experiment, but an appreciably greater amount of adrenaline was always liberated after eserine than before. In some experiments the quantity of adrenaline released was doubled: for example, in one experiment stimulation of the left greater and lesser splanchnic nerves for 20 sec. released just less than 5 μg. before eserine was administered. Afterwards, stimulation for the same time caused the liberation of nearly 10 μg.

RESULTS

Stimulation of the greater and lesser splanchnic nerves

Stimulation of these nerves invariably caused the liberation of adrenaline. The quantity released depended upon the strength and duration of the stimulus, and the condition of the animal. Well-nourished, friendly animals, which had been in the laboratory for a week or more, were always more satisfactory than those which had been in for only a few days. It was also observed that, in the winter, the amount of adrenaline liberated in experiments performed immediately after the week-end was small. This was possibly due to the lower temperature in the animal house during the week-end. The amount of adrenaline liberated in any one animal by a uniform stimulus remained fairly constant

during the course of an experiment of 3–4 hr. The retraction of the nictitating membrane following stimulation of the splanchnic nerves was matched by the intravenous injection of adrenaline in some experiments. The following figures, each of which is from a separate experiment, show the amounts of adrenaline liberated by stimulation of the greater and lesser splanchnic nerves together, at a frequency of 50 per sec. for 10 sec.; 1·0, 1·5, 3·0 and 20 μg. On the other hand, stimulation for 20 sec. in two other experiments liberated only 1·0 and 2·0 μg. respectively. After removal of one adrenal gland, no detectable adrenaline was liberated into the circulation, when the ipsilateral splanchnic nerve was stimulated (Fig. 1). In some experiments, eserine was given to

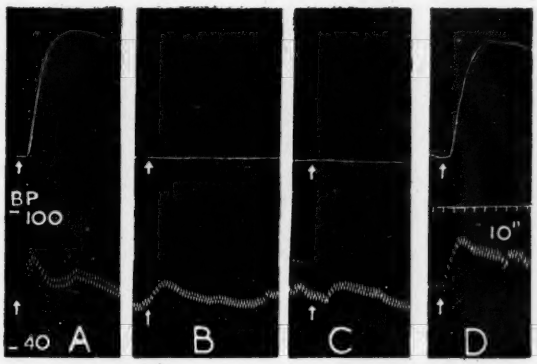


Fig. 1. Cat 1·75 kg. Spinal, eviscerated. Above: nictitating membrane. Below: blood pressure. A. Stimulation of left greater and lesser splanchnic nerves, 30 sec. B. Stimulation of left greater and lesser splanchnic nerves, 1 min. C. Stimulation of leash of fibres running from left to right coeliac ganglia. D. Stimulation of right greater and lesser splanchnic nerves, 30 sec. Between A and B, left adrenal gland removed.

sensitize the adrenal glands to the effect of splanchnic nerve stimulation: in these instances also, after removal of the gland, stimulation of the corresponding nerve did not cause the production of adrenaline by the opposite gland. In other animals, we attempted to demonstrate the presence of a crossed innervation of the adrenal medulla from the splanchnic nerves by stimulating the leashes of nerves running between the left and right coeliac ganglia. After removal of the one adrenal gland, one pointed silver wire electrode was passed through the ipsilateral coeliac ganglion, and the other hooked round the nerve-fibre bundles running to the opposite ganglion. In none of the animals, tested in this way, was the liberation of adrenaline observed, even after the administration of eserine. That the remaining adrenal gland contained adrenaline, and that its nerve supply was intact, was shown by the retraction of the nictitating membrane, and the rise in blood pressure after stimulation of the corresponding splanchnic nerves (Fig. 1).

Hollinshead (1936) states that in the cat the lesser splanchnic nerve usually

carries more of the fibres to the adrenal medulla than the greater splanchnic nerve. In several animals these nerves were stimulated separately. In most instances the effect of stimulation of the greater splanchnic nerve was conspicuously greater than that of stimulation of the lesser splanchnic nerve (Fig. 2). Occasionally the effects were almost identical (Fig. 3), but in no case was it observed that stimulation of the lesser splanchnic nerves liberated more adrenaline than that of the greater splanchnic nerve.

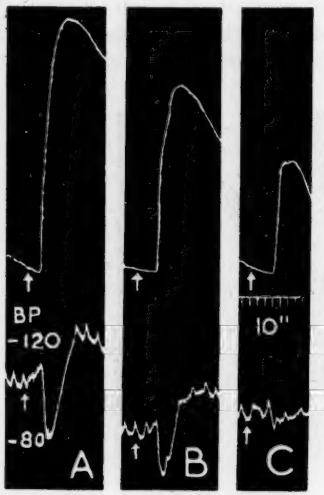


Fig. 2.

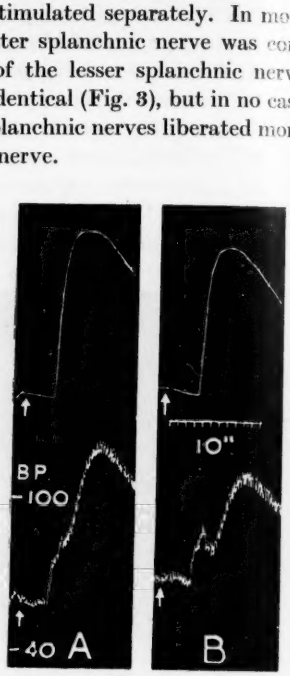


Fig. 3.

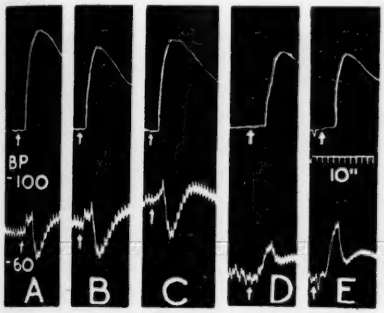
Fig. 2. Cat, 3·1 kg. Dial, eviscerated. A. Stimulation of left greater and lesser splanchnic nerves, 5 sec. B. Stimulation of left greater splanchnic nerve, 5 sec. C. Stimulation of left lesser splanchnic nerve, 5 sec.

Fig. 3. Cat. Spinal, eviscerated. A. Stimulation of left greater splanchnic nerve, 20 sec. B. Stimulation of left lesser splanchnic nerve, 20 sec.

Stimulation of the branches from the lumbar sympathetic chain

As stated above, the chain was divided between the last thoracic and first lumbar ganglia and between the third and fourth lumbar ganglia, and the grey and white rami communicantes reaching the ganglia through the psoas muscles were cut. In addition, the branches passing from the first, second and third lumbar ganglia to the pre-aortic area were crushed, proximal to the site of the electrodes, to minimize the possibility of stimulation of ganglion cells in the chain. Stimulation of the twigs running directly from the first and second ganglia almost always caused the liberation of adrenaline (Fig. 4). In some animals branches leave the sympathetic chain between the lumbar ganglia and run towards the root of the mesentery; usually not more than one is present between two ganglia. Stimulation of the branch leaving the chain between the first and second lumbar ganglia caused a discharge of adrenaline,

but no effect followed stimulation of the twig emerging from the chain between the second and third ganglia. In one animal no adrenaline secretion was obtained on stimulation of branches leaving the chains below the first lumbar ganglia. Removal of the ipsilateral gland abolished the effect of nerve stimulation, save in one animal. In this experiment, after removal of the left adrenal gland, stimulation of the branches running to the pre-aortic region from the second left lumbar ganglion still caused a liberation of adrenaline, but smaller than that obtained before removal of the adrenal gland. Removal of the right adrenal gland abolished this effect (Fig. 5).



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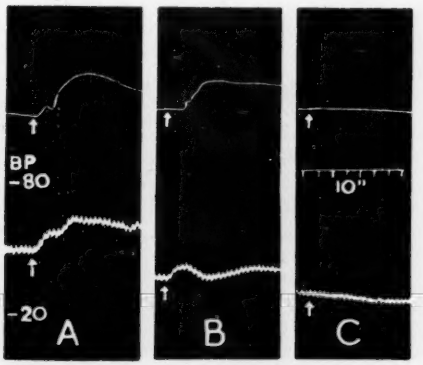

Fig. 5.

Fig. 4. Cat, 3·55 kg. Dial, eviscerated. A. 1·0 μg. adrenaline. B. 0·5 μg. adrenaline. C. Stimulation of left lesser and greater splanchnic nerves, 20 sec. D. Stimulation of branch from first left lumbar sympathetic ganglion 20 sec. E. Stimulation of branch from second left lumbar sympathetic ganglion, 20 sec.

Fig. 5. Cat, 3 kg. Spinal, eviscerated. A. Stimulation of branch from first left lumbar sympathetic ganglion, 10 sec. B. The same. C. The same, 15 sec. Between A and B left adrenal gland removed. Between B and C, right adrenal gland removed.

In all animals stimulation of the branches of the upper part of the lumbar sympathetic chain released an easily detectable amount of adrenaline. In some animals the amount of adrenaline liberated by stimulation of the branches from the first and second lumbar ganglia was only slightly less than that released by stimulation of the greater and lesser splanchnic nerves (Fig. 4). In Fig. 6 is shown an example from an animal in which the stimulation of these branches produced much less effect than stimulation of the splanchnic nerves.

In no animals was secretion of adrenaline observed after stimulation of the branches from the third lumbar ganglion even after the administration of eserine. In a small proportion of the experiments, however, stimulation of the chain between the second and third lumbar ganglia caused the release of adrenaline from the adrenal gland. Section of the chain immediately above

the second lumbar ganglion did not affect the result significantly, but, after cutting the branches from the second lumbar ganglion, stimulation no longer liberated adrenaline (Fig. 7).

Stimulation of the vagus nerves

Lewis & Gallo (1937) showed in dogs that, after sensitizing the adrenal gland to nerve stimulation with eserine, stimulation of the vagi would cause an inconstant liberation of adrenaline. In the present series of experiments, an attempt was made to repeat this result in cats after the administration of eserine. The peripheral ends of the cut vagi were stimulated in the neck, thorax and abdomen, but no evidence of the secretion of adrenaline was obtained.

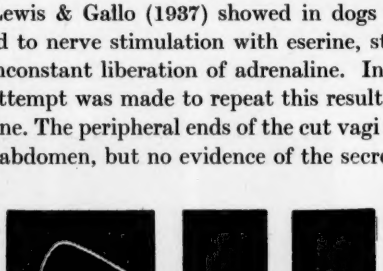

Fig. 6.

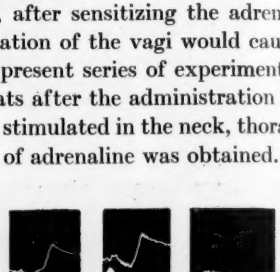

Fig. 7.

Fig. 6. Cat, 3·2 kg. Spinal, eviscerated. A. Stimulation of left lesser and greater splanchnic nerves, 20 sec. B. Stimulation of branch from first left lumbar sympathetic ganglion, 20 sec. C. Stimulation of branch of second left lumbar ganglion, 20 sec.

Fig. 7. Cat, 2·8 kg. Dial, eviscerated. A. Stimulation of left lumbar sympathetic chain between second and third lumbar ganglia. B. The same, after section of the chain between the first and second ganglia. C. The same, after section of the branch from second lumbar ganglion.

DISCUSSION

The evidence of various workers, gathered from histological investigation of the innervation of the cat's adrenal gland, is conflicting in many ways, and has usually not been accompanied by evidence of any function to support the conclusions drawn from the results of morphological study. There is general agreement that few or no nerve fibres can be traced to the cortex of the adrenal gland, and that all, save a few, of the fibres reaching the medulla are pre-ganglionic branches of the thoracico-lumbar sympathetic outflow, but there is great difference of opinion on the paths by which these fibres reach the adrenal medulla after leaving the anterior spinal roots. The results of the

experiments described above demonstrate that the adreno-secretory nerves of the adrenal gland in the cat are derived from the ipsilateral thoracico-lumbar outflow of the sympathetic nervous system. This conception has been generally accepted since the work of Elliott (1912), but the results of Nishi (1909), Kahn (1911) and Botar & O'Shaughnessy (1937) suggested that a bilateral nerve supply to the adrenal medulla might exist. Nishi and Kahn concluded that in rabbits the right splanchnic nerve supplied only the right adrenal gland, while the left splanchnic nerve controlled not only the left but also, to some extent, the right adrenal medulla. On the basis of histological studies, Botar & O'Shaughnessy concluded that the nerve supply was crossed and multi-segmental. Using the liberation of adrenaline as an indicator of effective stimulation of the nerves supplying the adrenal glands, no evidence has been found that the nerve supply to each adrenal medulla is derived from both sides of the thoracico-lumbar sympathetic outflow, save in one experiment out of a large number, in which an anomalous adreno-secretory pathway was traced from one lumbar sympathetic chain to the opposite adrenal gland.

The greater and lesser splanchnic nerves form the main pathway of adreno-secretory fibres to the adrenal glands, and usually the greater splanchnic nerve carries the preponderance of these fibres.

Adreno-secretory fibres also pass from the upper two lumbar sympathetic ganglia to the adrenal glands, and occasionally a contribution enters the chains at the third lumbar ganglia and ascends in the chains to the second lumbar ganglia, from which it passes to the adrenal glands. No evidence of the presence of adreno-secretory fibres in the vagus nerves was obtained in these experiments.

Young (1937) states that half of the nerve fibres reaching the adrenal medulla of the cat are carried in the greater splanchnic nerves, the other half passing through the lesser splanchnic nerves and branches of the upper lumbar sympathetic ganglia. There is no crossed innervation. Segmentally the fibres are derived from the seventh thoracic to the third lumbar anterior spinal roots: the outflow through the third lumbar anterior root is inconstant and was observed in one animal (Young, 1939). Hollinshead (1936) states that the number of fibres passing in the greater splanchnic nerves to the adrenal glands is negligible, the majority being carried in the lesser splanchnic nerves and branches from the lumbar sympathetic chains. The latter branches, in some cats, may carry a considerable portion of the total number of fibres reaching the gland. Botar & O'Shaughnessy (1937) could find no contribution from the lumbar sympathetic ganglia. The results described in this present communication agree closely with those of Young (1937), and support Hollinshead's (1936) statement that in some animals the contribution from the lumbar sympathetic chains is of great importance. But no physiological evidence has been obtained that the greater splanchnic nerves carry only a negligible number of adreno-secretory fibres, as Hollinshead suggests.

In all the animals examined, the greatest liberation of adrenaline has been

obtained by stimulation of the greater splanchnic nerve. Sometimes the effect of stimulation of the lesser splanchnic nerve has been almost as great as, but never greater than, that of stimulation of the greater splanchnic nerve. The magnitude of the adreno-secretory effect on stimulation of the branches of the upper lumbar ganglia, as judged by the liberation of adrenaline, varies. In the experiments reported here, however, stimulation of these nerves has usually produced an easily detectable outpouring of adrenaline, occasionally almost as great as that evoked by stimulation of the splanchnic nerves themselves. In experiments in which denervation of the adrenal medulla is desired, it is, therefore, not sufficient merely to cut the greater and lesser splanchnic nerves on each side, or to remove one gland and cut both splanchnic nerves supplying the contralateral gland. To ensure complete denervation, it is necessary to remove the upper three lumbar sympathetic ganglia on each side, as well as to cut the greater and lesser splanchnic nerves on each side.

SUMMARY

The distribution and relative importance of the adreno-secretory nerves of the adrenal medulla have been investigated in the cat, using the retraction of the denervated nictitating membrane as an indicator of the adrenaline released by nerve stimulation.

The nerve supply of the adrenal medulla is derived from the ipsilateral thoracico-lumbar sympathetic outflow.

Most of the adreno-secretory nerves are carried in the greater and lesser splanchnic nerves. Some also run to the adrenal medulla from the first and second lumbar sympathetic ganglia. The relative importance of these paths varies from animal to animal. Occasionally adreno-secretory nerves may enter the lumbar sympathetic chains at the third lumbar ganglia and ascend to the second lumbar ganglia, whence they pass to the adrenal glands.

No release of adrenaline was obtained by stimulation of the vagus nerves.

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DISCONTINUITY IN THE NERVOUS SYSTEM OF COELENTERATES

By the late H. H. WOOLLARD and J. A. HARPMAN

From the Institute of Anatomy, University College, London

INTRODUCTION

The occurrence of nerve nets in such places as the skin, the tunica media of the blood vessels and the enteric plexuses in vertebrates is frequently unquestioned by reason of the prevalent notion that the simplest invertebrates possess a nervous system in which there is syncytial continuity between the nerve elements. Recent unpublished investigations in mammals have thrown doubt upon the existence of such nerve nets. It therefore seemed advisable to restudy the primitive nervous system in coelenterates.

The extensive literature relating to the nervous system of coelenterates has been summarized by Bozler (1927) and Heider (1927). The histology of the coelenterate nervous system was first investigated by the Hertwigs (1878, 1879, 1880) and by Schäfer (1878), following up the experimental work of Romanes (1876, 1877). The former worked on Medusae and Actiniae and were non-committal with regard to continuity, but Schäfer, who worked on the common jelly-fish, *Aurelia aurita*, was definite that the nerve fibres were discontinuous. The succeeding papers dealing with this problem were consistently in favour of the continuity of the nerve fibres and therefore of the existence of a true nerve net. However, Bozler (1927), describing the nervous system of the large jelly-fish *Rhizostoma*, concurred with the views of Schäfer, and mentioned that few investigators appear to have appreciated the important work of the latter. Heider (1927), working on ctenophores, and McConnell (1932), working on *Hydra*, are the most recent investigators who claim evidence for the idea of continuity.

OBSERVATIONS

Material. The forms studied included the sea-anemones *Tealia felina* and *Actinia equina*, and the jelly-fishes *Cyanea lamarcki* and *Chrysaora isosceles*.

Methods. Whole animals or fragments from the subumbrellar ectoderm of the jelly-fishes were placed for $\frac{1}{2}$–2 hr. in 0·1–0·05 % methylene blue in sea water. The tentacles of the sea-anemones were studied similarly, as well as by the gold chloride-formic acid method. The jelly-fish material was studied in the living, since like other workers we found the preservation of these animals very unsatisfactory.

Findings. Nerve cells are scattered all through the plexuses and as many as five or six may be present in a 1/12th oil-immersion field. They are mainly bipolar; a few are multipolar. In the bipolar type the nerve processes start from opposite ends of the ovoid cell body. The cell body is small, and its small size seems out of proportion to the size and length of the processes. The nucleus is small and compact. It often shows the characteristic nucleolus of the mammalian nerve cell. With methylene blue staining, granules are demonstrable in the cytoplasm of the cell and also in the basal part of the processes. Groselj (1909) thought that these granules might represent tigroid substance. In none of the regions studied are the nerve cells gathered together in ganglionic masses.

The fibres show no structural differentiation. Myelin sheath and neurilemma are both absent, and there is nothing to suggest any structural difference indicating conduction to or from the cell. Either process (or, where there are several, any or all of them) may subdivide. Some fibres are thick and others thin. On tracing individual fibres it can be seen that the same fibre may begin as a thick one, but as it extends it grows thinner, eventually becoming so thin as to pass beyond the powers of resolution of the microscope. At their origin from the cells the fibres are all of about the same thickness. The diameter, therefore, would seem rather a function of the distance travelled by the fibre than a distinction in form and an indication of different physiological properties.

Many of the processes have a varicose appearance; irregular swellings occur at varying intervals. When portions of tissue are immersed for several hours in the methylene blue, the swellings become very large. It is tentatively concluded that the prolonged action of the methylene blue leads to a loss of viability of the tissues and thus to a pathological increase in size of the varicosities. Small irregular thickenings are demonstrated by gold chloride impregnation (Pl. I, fig. 3), and can be seen in methylene blue staining before viability is appreciably altered.

The direction pursued by the fibres appears at first sight quite irregular, but on further observation many are seen to run towards the muscular layer (Pl. I, figs. 1, 2). Round pigment cells are scattered singly or clustered in groups. The nerve cells are seen as small ovoid swellings interposed in the course of the fibres (figs. 2, 3). Single fibres can be traced for relatively long distances. They may take a straight or a sinuous course. Some do not branch, while others soon subdivide. As they approach the muscle many of the nerve fibres become somewhat thinner. Upon reaching the muscle fibres they undergo subdivision into a number of very small branches which end upon the surface of the muscle cells by means of small expansions. This pericellular investment appears to be similar to the nerve endings that occur in non-striated muscle in mammals.

Where continuity has been described in the coelenterate nervous system, this has been represented as an end-to-end fusion between the fibres. That such a simple arrangement does not occur is seen in Pl. I, figs. 1, 2. Although

at low magnifications it seems possible that fusion between the nerve fibres occurs where these cross one another, this doubt can be dispelled by the use of high-power objectives (Pl. I, fig. 3). At such points of crossing one of the fibres sometimes shows a thickening. No fusion, however, occurs.

The fibres frequently present a characteristic relation to one another, that of intertwining. The fibres may wind round one another once (figs. 1, 2 and 4) or several times (fig. 1). At these intertwinings no fusion occurs, each fibre retaining its individuality. Schäfer was also impressed with the frequency with which fibres intertwined and suggested that such an arrangement presented the opportunity for "induction" to occur. Fibres have also been observed to lie in close contact with the surface of nerve cells. Sometimes a structure that possibly represents a "bouton" can be observed.

Many neuro-sensory cells have been seen, especially in the methylene blue material. They correspond in every detail with the careful descriptions of such cells that have been given by Groselj. These neuro-sensory cells give off a process from their deep aspect, but this conducting process we were unable to trace far.

COMMENT

It is permissible here to refer to the recent physiological investigations of Pantin (1935) on various coelenterates. In addition to other observations, Pantin showed that the nervous system of sea-anemones possesses some degree of polarization and facilitation. The reactions are diffuse and the frequency of "after-discharge" complicates considerably the interpretation of what Pantin believed from the literature to be a nerve net. It appears to us that a nervous system such as our observations lead us to believe occurs in the coelenterate would account more satisfactorily both for the diffuseness of response and for the other properties, viz. facilitation, polarization and "after-discharge" described by Pantin.

Certain histologists (Boeke, 1935; Stöhr, 1928; Rieser, 1933; etc.) believe that in vertebrates neural syncytia (variously described as the sympathetic ground plexus, the periterminal network, and the terminal reticulum) occur throughout the body. Such a conception, however, cannot be supported by any appeal to more primitive types of nervous systems. Their existence and their significance must be proved by investigations upon the animals in which they are said to occur.

The existence of a neurencytium amidst an otherwise synaptic system in higher animals would be surprising in the light of the occurrence of discontinuous neurones in primitive forms.

SUMMARY

1. The histology of the nervous system in the sea-anemones *Tealia felina* and *Actinia equina*, and the jelly-fishes *Cyanea lamarcki* and *Chrysaora isosceles*, studied by the methods of methylene blue and gold chloride, is described.

2. It is concluded that the nervous system in these coelenterates consists of nerve cells the processes of which are discontinuous.

3. The nerve fibres frequently intertwine but never fuse.

4. Nerve fibres terminate on muscle cells by small expansions.

We are indebted to Dr Stanley Kemp, Director, and the staff of the Marine Biological Association Laboratory at Plymouth for the generous help and facilities which they have given us.

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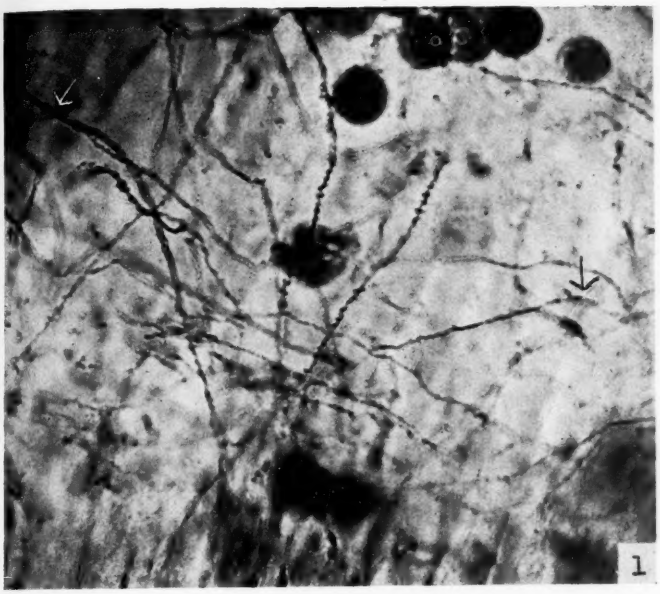
EXPLANATION OF PLATE I

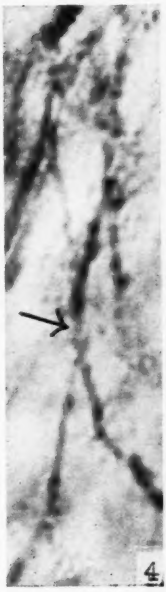
Fig. 1. Photomicrograph showing the nerve plexus in a tentacle of *Actinia equina*. The two arrows indicate intertwinements between fibres. In the lower part of the figure some of the nerve fibres are seen to enter the muscle layer. Gold chloride. ×900.

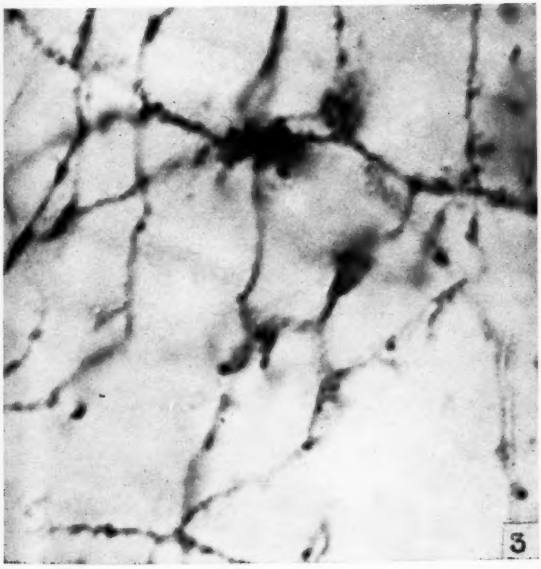
Fig. 2. Photomicrograph showing the nerve plexus in a tentacle of *Actinia equina*. In the upper part of the figure nerve fibres are seen to course in among the muscle fibres. In the centre of the picture a group of nerve cells is seen. In the lower part of the figure an arrow points to an intertwinement between fibres. Gold chloride. ×900.

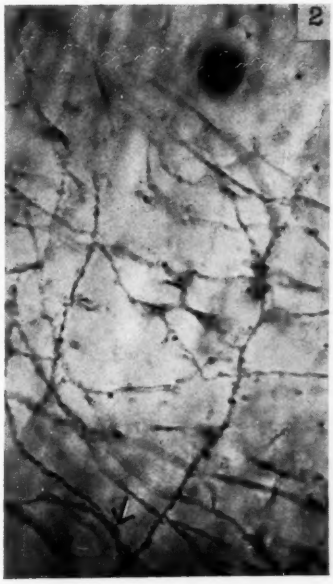
Fig. 3. A portion of the plexus illustrated in fig. 2 at a higher magnification, showing nerve cells and the varicose appearance of the fibres, which cross each other but do not fuse. ×2200.

Fig. 4. Photomicrograph showing an intertwining between nerve fibres in a tentacle of *Tealia elina*. An arrow points to the position of intertwinement. Gold chloride. ×1550.









UNIV. OF MICH.

STRUCTURAL CHANGES IN THE ISOLATED VISUAL CORTEX

By W. E. LE GROS CLARK and S. SUNDERLAND
Department of Human Anatomy, Oxford

There is still lack of agreement on the question of the functional significance of the laminar differentiation of the cerebral cortex. If functional localization referable to the cortical cell laminae does occur, it must presumably be reflected in a difference in their fibre connexions. This, again, should be demonstrable by a study of the changes in the cell and fibre architecture of the cortex which may occur after interruption of its connexions with other parts of the cortex and with subcortical centres. In this paper is recorded an experimental study of the visual cortex in the monkey (*Macaca*) to ascertain whether this is the case. The visual cortex is particularly suitable for such an investigation, partly because the lamination here is very well differentiated, and partly because recent work has established in considerable detail the organization of the striate area in relation to subcortical centres.

In these experiments small areas of the visual cortex have been completely isolated without interference of their vascular supply in order to determine, primarily, the laminar distribution of cells concerned with efferent functions (whether related to neighbouring or distant areas of the cerebral cortex, or to subcortical centres), and also the alteration in fibre architecture consequent on the interruption of afferent connexions.

LITERATURE

Current ideas regarding the allocation of specific functions to particular cell laminae generally reflect the conclusions drawn by Kappers (1909) from morphological studies of the neocortex. Kappers supposed that the cortex can be divided into three functionally different laminated zones: a supragranular (superficial to Brodmann's lamina IV) which is essentially receptor-correlative in function, an infragranular which gives origin to corticofugal and commissural fibres, and between the two a granular zone (Brodmann's lamina IV) which is predominantly receptive. A good deal of evidence has been adduced in support of this conception. For example, it has been noted that the granular layer is best developed in cortical areas which are known to be sensory in function, and is absent or poorly developed in motor areas. The allocation of receptive functions to this layer is also substantiated by the evidence of Marchi preparations. After section of afferent connexions, the degenerating fibres can be seen to run up into the granular layer and there break up into a fine

deposit of Marchi granules (see, for example, Poliak, 1927, 1932). This appearance, however, does not preclude the possibility that many of the fibres may terminate (either directly or by collaterals) in the infragranular zone, or that others, after losing their myelin sheaths, may extend into the more superficial layers of the cortex. Lorente de Nó (1923), in his study of Golgi preparations of the cortex in the mouse, found relatively coarse fibres (which emerged from the corona radiata) entering lamina IV without giving off any significant numbers of collaterals to deeper layers. On the other hand, however, he also traced finer fibres to the pyramidal layer and even to the superficial lamina zonalis, some of which do give off numerous collaterals to laminae V and VI. According to a more recent statement by Lorente de Nó (1938), the cells of lamina IV certainly have the greatest number of synapses with specific afferent fibres, while other fibres (believed to have their origin in the thalamus) ascend as far as the lamina zonalis, giving off collaterals to other laminae in their course through the thickness of the cortex. A third type of afferent path is represented by association fibres which give off collaterals in the deep layers, but whose main territory of distribution is laminae I to IV, and especially II and III. O'Leary & Bishop (1938) arrived at somewhat similar conclusions from a study of the visual cortex of the rabbit; they found that the afferent fibres end predominantly in lamina IV and the lower two-thirds of lamina III.

As regards the associative functions of the supragranular layers, it has been pointed out that these are exceptionally well developed in certain of the so-called "association" areas, they are on the whole relatively better developed in more highly organized brains, while, according to Bolton (1900), they are the main site of pathological cell involvement in the cortex in conditions of amentia and dementia. It has been mentioned above, also, that Lorente de Nó's observations led him to infer that association fibres reaching an area of the cortex are distributed mainly to the lamina pyramidalis.

Nissl (1911) found that cell atrophy in the cortex following separation of the hemispheres from subcortical centres in young rabbits is most marked in the infragranular layers, while the superficial layers show little change, or even retain the capacity for further development. This observation is in accord with the generally accepted opinion that the infragranular zone gives rise to corticofugal pathways of all types. It is, of course, well established that in the motor cortex the cells of origin of the pyramidal tracts are situated in the infragranular layers. As far back as 1909 this was determined experimentally by Holmes & May. These authors divided the pyramidal tract in the upper cervical region of the spinal cord in the cat, dog, lemur, monkey and chimpanzee, and found the resulting cell atrophy in the motor cortex to be localized to the giant cells of Betz. So far as the other cells are concerned, they write, "we never succeeded in detecting chromatolytic changes in the superficial pyramids, in the polymorphous layer, or in any of the smaller pyramids or granule cells". On the other hand, following a capsular lesion (in man) and a

lesion in the crus cerebri (in the lemur), additional changes were observed in the pyramidal cells of the supragranular and infragranular layers, but especially the latter. In a recent paper, Levin & Bradford (1938) have reported similar findings after experimental section of the pyramidal tract in monkeys. Seventeen days after operation, they found that chromatolysis of the Betz cells had for the most part become fully developed, and after 28 days most of the degenerating cells had already disappeared. These authors also found no evidence of cell change in the supragranular layers. The relation of the infragranular layer of the motor cortex to the pyramidal tract has been further substantiated by the thermo-coagulation experiments of Dusser de Barenne (1934). Lastly, there is some evidence (van Valkenburg, 1913; de Vries, 1912), based on retrograde cell degeneration, that callosal fibres take their origin chiefly from pyramidal cells of the infragranular layers of the cortex, and terminate in the cortex of the opposite hemisphere mainly in relation to the granular layer.

In general, it may be said that the recent work of Lorente de Nó on cortical organization has thrown considerable doubt on any rigid conception of a laminar type of functional localization in the cortex, for his observations have shown that it is possible for cells in any layer to have synaptic connexions with afferent fibres. In every layer, also, there are cells giving origin to descending axones which reach the white matter. These conclusions, however, do not eliminate the possibility that the different cell laminae may each be predominantly concerned with different functions.

METHODS

In the experiments recorded in this paper, the choice of the operative technique employed to isolate cortical areas has taken account of the fact (Sunderland, 1938) that the cortex and the immediately subjacent white matter receive their entire effective blood-supply from the superficial pial vessels. In three monkeys, a strip of cortex along the margin of the occipital operculum (containing portions of area 17 and area 18) was isolated by means of a curved sub-pial incision with a Graefe knife, care being taken to avoid injury to the superficial vessels passing to the isolated area (Text-fig. 1). In one animal (O.M. 164), a small rectangular area of cortex in the central part of the occipital lobe was also isolated by incisions the position of which is indicated diagrammatically in Text-fig. 1. Of the three animals, one (O.M. 149) was allowed to survive for only 14 days after the operation, to allow of the study of chromatolytic changes in affected cells before total atrophy had occurred. Of the others, one (O.M. 164) survived for 46 days, and a third (O.M. 226) for 118 days. After such lengths of time, atrophy of cells giving rise to efferent fibres should be complete.

After the brains had been removed at death, suitable blocks containing the isolated areas of cortex were prepared, while corresponding areas from the

normal hemisphere were also removed for purposes of comparison. The blocks were fixed in 90% alcohol containing 2% acetic acid, and embedded in paraffin. Sections were cut at 15 μ; some of these were stained with Borrel's methylene blue, and others by Bodian's silver technique.

In order to give some indication of the extent of the isolated cortex, it may be noted that the maximal width of the isolated opercular lip was 7 mm. in O.M. 149, 4 mm. in O.M. 164, and 6 mm. in O.M. 226. The maximal diameter of the isolated area in the central part of the occipital lobe in O.M. 164 was 8 mm.

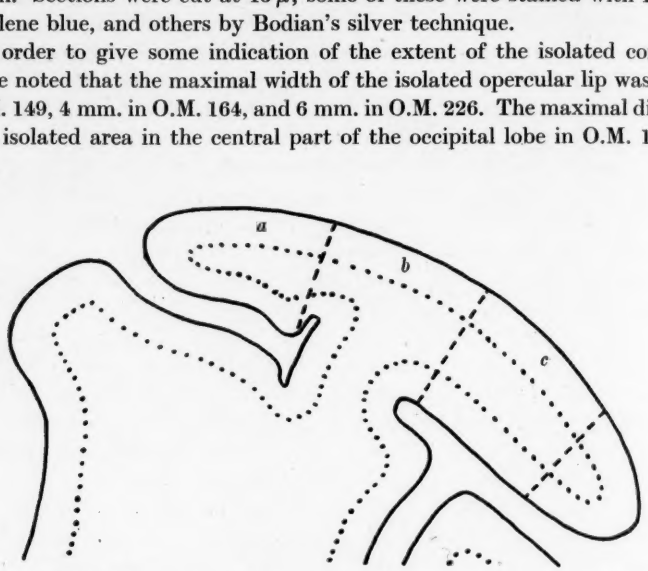


Fig. 1. Diagram of a horizontal section through the occipital lobe of the brain of a monkey to show the position of the sections made in the cortex. *a*, the opercular lip, which was separated from the rest of the cortex in three experiments. In one experiment (O.M. 164), an area situated more posteriorly was also isolated, *c*. Between *a* and *c* an area of cortex *b* was left which still retained its connexion with the medullary white matter of the occipital lobe while separated in front and behind from the neighbouring visual cortex.

OBSERVATIONS

Before discussing the structural changes which ensue in an isolated area of visual cortex, certain features of its normal histological structure require a brief mention. The general structure of the visual cortex of Primates has been described in detail by Brodmann (1903), Beck (1930), Ngowyang (1935, 1937) and others. Beck and Ngowyang, in particular, have shown that the area striata of the cortex may be subdivided into a number of subsidiary cytoarchitectural fields; this fact is of considerable importance in the present study, since it makes it necessary that, for comparative study, the isolated sections of the cortex and the normal control should be taken from identically equivalent areas in order to avoid errors related to regional variations.

In the lamina pyramidalis of area 17, well-formed pyramidal elements are relatively inconspicuous in methylene blue and silver preparations; they show considerable variation in size, and they are not disposed in layers as they are in adjacent cortical areas. In area 18, on the other hand, one of the most

characteristic features is the conspicuous number of pyramidal cells which occupy mainly the deeper levels of the lamina pyramidalis.

In the visual cortex of the monkey's brain, the large cells of Meynert in lamina VI present a very striking appearance (Pl. II, fig. 7). In size, their cell body may reach a diameter of 45 μ. Some are pyramidal in shape, but many are irregularly angular, with numerous stout dendrites extending for considerable distances in a horizontal direction. In some cases an apical dendrite can be traced up as far as the pyramidal layer, and an axonal process descending into the white matter. These cells tend to be arranged in a single linear series, and they vary considerably in number in different sections. They are often quite widely spaced, so that in a single section adjacent cells may be separated by an interval of several millimetres.

In lamina IV*b* (the stria of Gennari), large stellate or pyramidal cells are present in large numbers, and in some regions of the visual cortex (particularly the central and posterior parts) they are arranged in a fairly well-defined band (Pl. II, fig. 5). These large cells were also originally described by Meynert (1872) as characteristic of the visual area of the cortex. In the monkey they are much more conspicuous than in Man, but, though large, they do not reach the size of the solitary cells of Meynert in lamina VI. In silver preparations they can be observed to give off apical and horizontal dendrites.

Changes in the isolated cortex. It may be stated at the outset that perhaps the most striking feature of the isolated visual cortex is the extent to which its cytoarchitecture persists unchanged. This can be seen by reference to Pl. I, figs. 3, 4, which show a microphotograph of the opercular lip from the normal hemisphere of O.M. 226, compared with the operated side in which the opercular lip had been isolated from the rest of the occipital cortex for 118 days. At first sight, indeed, the cytoarchitecture appears hardly to be affected, the only noteworthy feature being the gliosis in the medullary white matter of the isolated area.

Attempts were made at a numerical computation of cells in normal and isolated areas of O.M. 164 and O.M. 226 in order to determine the extent of total cell atrophy. This was done by taking microphotographs of sections (stained with methylene blue) and counting directly the cells in random areas of different laminae. Such estimates presented considerable difficulty, partly because of the absence of a clear line of demarcation between the various layers, and partly because it was not possible to take into account any slight local shrinkage which may have affected the cortex as a whole. Furthermore, there remains the possibility that the initial vascular disturbance following immediately on the operative interference may have led to degenerative changes in some of the cortical cells, even though their direct blood supply was not interrupted. In O.M. 164 (killed 46 days after operation) a definite reduction of cells was demonstrated by this method and is, indeed, directly evident by a comparison of Pl. II, figs. 5, 6. It is interesting to note, however, that the reduction has involved all the cell laminae to some degree, amounting

to 15–20 % in the supragranular and granular layers, and not more than 6 % in the infragranular layers. Such a result is hardly in accordance with the general assumption that the infragranular layers consist predominantly of efferent neurones. It should be pointed out, however, that, in so far as callosal fibres may take their origin from the infragranular layers, some of the reduction of cells in the latter may be masked by the fact that a similar reduction would also have occurred in the visual cortex of the normal (contralateral) hemisphere. In O.M. 226 (which survived 118 days after operation) the reduction of cells as estimated by the methods employed appeared to be very much less, amounting to 7 % in the supragranular layers, 9 % in the infragranular layers, while there was no apparent reduction in the granular layer. In this case, however, there seems to be some reduction in the thickness of the cortex as a whole, consequent on atrophic shrinkage, but unfortunately the degree of shrinkage cannot be estimated with any degree of accuracy because of the possibility that the sections may not have been cut in a plane precisely vertical to the surface. Our conclusions from these observations are that, in the isolated visual cortex, there is certainly some reduction of cells, but that this affects no particular lamina to the exclusion of others. We may now refer to the cell changes which occur in individual laminae of the isolated cortex.

Lamina pyramidalis. After 14 days, no changes in the pyramidal cells were observed; medium-sized pyramids of quite normal appearance are present in their usual numbers. After 46 days there is a reduction in number, but no abnormal appearance is presented by the remaining cells, except that many of them stain less deeply and less sharply. After 118 days little further change has occurred; the larger cell elements of the pyramidal layer appear less conspicuous in number, and some of them have taken on a vesicular appearance.

Lamina granularis. Brodmann's lamina IV is divided into three layers in the visual cortex. Of these, the outermost (IV *a*) is so difficult to separate topographically from the pyramidal layer that we found it impracticable to consider it separately. Lamina IV *b* contains the large stellate cells to which reference has already been made, and these elements show very definite changes following isolation of the cortex. After 14 days they are still easily recognizable, but, while the majority of them appear quite normal cytologically, some give the appearance of undergoing chromatolytic changes, with eccentric nuclei, vacuolation of the cytoplasm, and less clearly defined cell processes. After 46 and 118 days, most of the large stellate cells have disappeared (see Pl. II, figs. 5, 6). A few persist unchanged, but even these do not stain with their normal sharpness. Bodian sections give a rather better contrast in some respects, for they demonstrate more clearly the relatively complete absence in lamina IV *b* on the operated side of the characteristic large cells with branching processes.

It should be noted that, in O.M. 164, while the large stellate cells have mostly disappeared in the isolated areas marked *a* and *c* in Text-fig. 1, they

remain apparently unaffected in area *b*. It thus appears probable that their disappearance is consequent on the interruption of fibres which run into the medullary white matter of the occipital lobe, and not on the interruption of association fibres passing to the immediately adjacent cortex.

Infragranular layers. Apart from a general small reduction of cells in these layers of the isolated cortex, the most striking change is that which affects the large solitary cells of Meynert. After 14 days these cells show significant changes. In Nissl sections some of them present a chromatolytic appearance; many of them, however, are still quite normal in their cytological characters though they are smaller. This shrinkage is more clearly demonstrated in Bodian sections (Pl. II, figs. 7, 8). Here the alteration in size is quite evident, the shrunken cells in the isolated cortex also staining more densely. After 46 and 118 days, many of the cells have undergone complete atrophy, leading to a reduction in number of approximately 50 %. Those that remain are small; in no case has it been possible to find large branching Meynert cells of the type seen in normal visual cortex. It may be observed, again, that in O.M. 164, while typical Meynert cells have disappeared from the isolated areas *a* and *c*, some were still present in areas whose subcortical connexions had not been interrupted.

Discussion of cell changes in the isolated visual cortex. We have already emphasized the small extent of the change in the cytoarchitecture of a completely isolated area of the visual cortex. This may be taken to indicate how rich must be the intracortical connexions of any point on the cortex with the immediately adjacent region of the cortex, since it is reasonable to expect that neurones which are entirely concerned with efferent connexions to more distant parts of the visual cortex, to other cortical areas, or to subcortical centres, would undergo rapid degeneration after interruption of their axonal processes (as occurs, for example, with the Betz cells in the motor cortex). It seems evident from our experiments that there is a small reduction of cells affecting all the laminae of the isolated visual cortex, but we have found no certain evidence that any particular cell lamina is more concerned with efferent functions than others. Such a conclusion is in general accord with the observations of Lorente de Nó on the organization of the cerebral cortex. However, this statement may be qualified by reference to the large stellate cells in lamina IV *b*, and the large cells of Meynert in lamina VI. The experiments have shown that most of the former and many of the latter undergo complete atrophy in isolated areas. It may be inferred, therefore, that they are predominantly concerned with giving origin to efferent connexions. The question now arises whether these are relatively short connexions passing to the adjacent areas of the visual cortex, longer association fibres passing to the parietal cortex, or subcortical projection fibres. That they are not fibres of the first category is indicated by the observations already recorded in the experiment O.M. 164, for they persist in their normal numbers in a small area of cortex separated in front and behind from other parts of the visual cortex,

but still retaining its connexion with the medullary white matter of the occipital lobes. That they do not project on to the parietal cortex is shown by the results of an experiment reported elsewhere (Clark & Northfield, 1937) in which the whole of the parietal lobe of the brain was removed in a monkey 2 months before the animal was killed. In this case, a careful study of the cytoarchitecture of the visual cortex failed to show any recognizable change—large stellate cells and Meynert cells were still present, apparently in their normal numbers. It appears, therefore, that these cells are concerned with subcortical efferent projection, and it may be suggested that these include the corticotectal connexions of the visual cortex.

The absence of demonstrable changes in the cytoarchitecture of the visual cortex following ablation of the parietal cortex is somewhat remarkable in view of the general assumption that the two are closely linked by association fibres. However, Poliak's studies on the monkey's brain (1932), with the use of the Marchi technique, have indicated that association fibres arising in the visual area are mainly dispersed to the neighbouring portions of the visual cortex itself, some also reaching the immediately adjacent segments of areas 18 and 19, but not extending to other cortical areas in the parietal lobe.

Structural changes in area 18. It has been mentioned that a small portion of area 18 (area peristriata) was included in the isolated regions of the opercular lip of the occipital cortex. The cytoarchitectural changes here affect mainly the large pyramidal cells in the deeper levels of the lamina pyramidalis. Many of these undergo complete atrophy and disappear, especially those that lie close to the margin of the area striata. On the other hand, large numbers of conspicuous pyramidal cells remain in this small strip of cortex unchanged, even after it has been isolated for a period of 118 days (Pl. I, fig. 4).

Changes in the fibre architecture of the isolated visual cortex. The fibre architecture of the cortex was studied in the Bodian sections. As with the cytoarchitecture, a striking feature is the relatively small degree of change which follows isolation. For purposes of description, three categories of fibres may be recognized in the cortex, (1) radial fibres which run vertically through the lower levels of the cortex in discrete fasciculi; in sections cut at 15 μ there are between fifty and sixty of these fasciculi in each millimetre, (2) a diffuse network of obliquely disposed fibres of varying calibre, which are not collected in discrete bundles, and (3) the closely meshed felt-work of fibres which forms the basis of the stria of Gennari.

With regard to the radial fasciculi, comparative study of the isolated and normal visual cortex shows that, although the number of fasciculi remains unchanged, there is some thinning out of individual fibres (Text-fig. 2). With

LEGEND TO FIGURE 2

Fig. 2. Sections of the normal (A) and isolated (B) cortex of O.M. 226, stained by Bodian's silver technique. The sections include lamina IV c, with a portion of the stria of Gennari above, and lamina V below. Note that in the isolated cortex the background of interlacing, obliquely running fibres has largely disappeared, while there is also a reduction of fibres in the individual radial fasciculi. × 350.

Fig. 2.

an ocular micrometer and the use of a 1/12th oil immersion lens, actual counts of fibres were made in fifty different fields of the normal and isolated cortex in O.M. 226. These showed that in a length of normal cortex of 80 μ there was an average of twenty-four fibres, while on the operated side the average was 20·5. There appears, therefore, to be a reduction in the isolated cortex of rather less than 15 %. It is interesting to note that in O.M. 164, this reduction affected not only the isolated cortex of the opercular lip, but also the area *b* (shown in Text-fig. 1) which had been separated on either side from the adjacent visual cortex while still retaining its connexions with the medullary white matter of the occipital lobe. It thus appears that the radial fasciculi are made up of association fibres connected with adjacent regions of the cortex and not of projection fibres connected with subcortical levels. This is in accordance with the observations of Poliak (1932) in his studies of Marchi degeneration in the cortex of the monkey's brain following capsular lesions. Lastly, it may be noted that the reduction of the radial fibres affects mainly those of larger calibre, and that it involves no reduction in the number of the radial fasciculi in a given length of cortex.

Perhaps the most evident change in the fibre architecture of the isolated cortex is the disappearance of a large proportion of the obliquely running fibres in the lower levels of the cortex. This is made evident in the microphotographs shown in Text-fig. 2 in which the "background" of lamina IV *c* is manifestly much clearer in the isolated cortex. This reduction of the obliquely disposed fibres (particularly affecting the axones of larger calibre) is not present in area *b* of O.M. 164, which suggests that they are predominantly composed of projection fibres reaching the cortex from subcortical levels, rather than association fibres from neighbouring regions of the cortex. This, again, is in accord with Poliak's observations noted above.

The stria of Gennari appeared to be unaffected by isolation; we were unable to discern any alteration in the thickness of this stria or in its density. This is somewhat surprising in view of the statements made by several observers that changes in the area striata following on prolonged blindness take the form of a reduction of the stria. Bolton (1900), for example, maintained that in congenital anophthalmos the stria becomes narrowed to two-thirds of its normal thickness. Poliak (1927) has also stated that in the cat the stria does not originate in the cortex itself; in other words, it is not an endogenous association layer but an exogenous projection system. However, he qualifies this statement by observing that "this view does not conflict with the existence of ramifications of other axis cylinders in the same stratum, fibres which originate in the cells of the visual cortex itself". Our own observations suggest that, in the monkey, the stria of Gennari is predominantly composed of endogenous fibres.

SUMMARY

1. The structure of an isolated area of visual cortex shows relatively little change in its cell or fibre architecture. This is taken to indicate the richness of the intracortical connexions of each part of the cortex with the immediately neighbouring cortex.

2. In the isolated cortex there is a slight general reduction of cells which affects all the laminae. Most of the large stellate cells of lamina IV *b* and many of the large solitary cells of Meynert in lamina VI undergo atrophy and disappear. Evidence is adduced that these cells give origin to projection fibres passing to subcortical centres, rather than to association fibres either to neighbouring or distant areas of the cortex.

3. In the isolated cortex there is a reduction of the number of individual radial fibres, while the number of fasciculi which they compose remains unchanged. It is probable that these radial fibres are association fibres derived from other parts of the visual cortex. The network of obliquely disposed fibres in the deeper levels of the cortex mostly disappears; the experiments suggest that these fibres are projection fibres derived from subcortical centres. The stria of Gennari remains apparently unchanged in width and density.

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EXPLANATION OF PLATES I AND II

Plate I

Fig. 3. Section through the opercular lip of the normal visual cortex of O.M. 226. ×35.

Fig. 4. Section through the isolated opercular lip of O.M. 226. ×35. These sections demonstrate the small degree of alteration in the cytoarchitecture of the visual cortex even after it has been isolated for a period of 118 days.

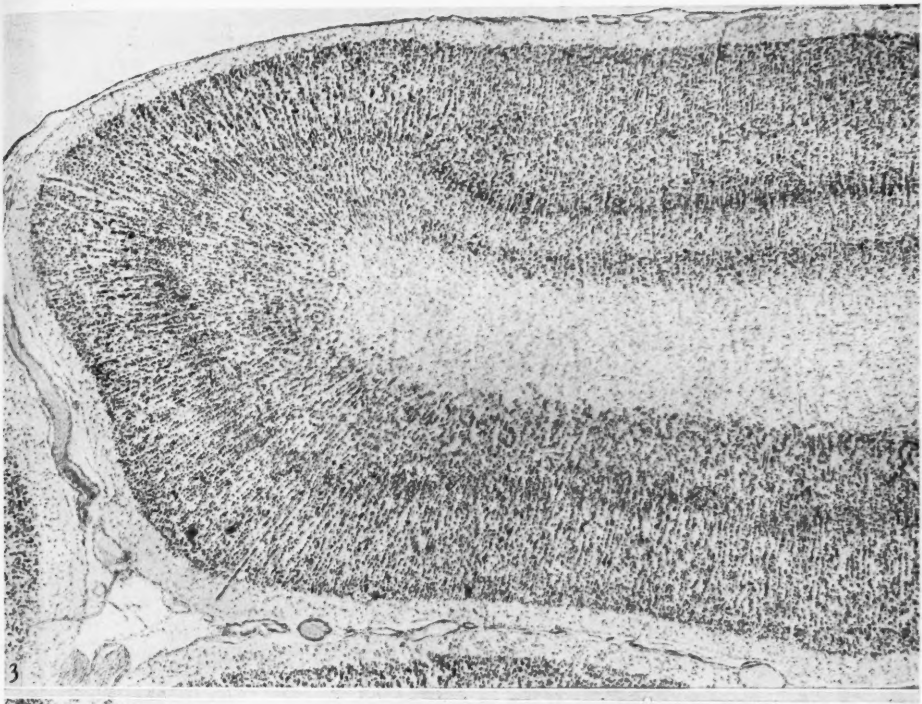
PLATE II

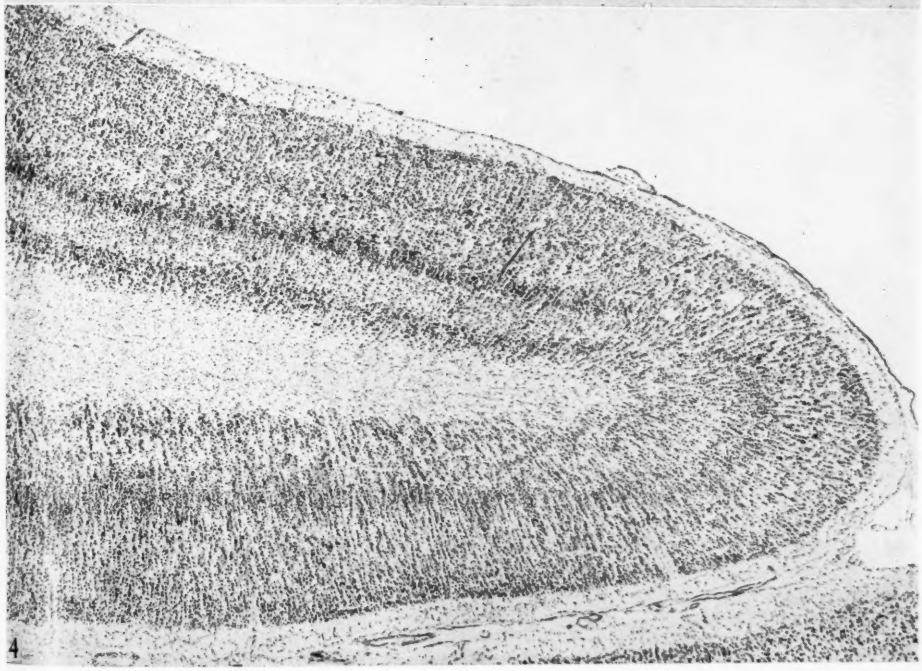
Fig. 5. Section through the normal visual cortex of O.M. 164, taken from the central region of the occipital lobe. ×72.

Fig. 6. Section through an isolated area of visual cortex from a corresponding region of the occipital lobe (area *c* in Text-fig. 1), showing disappearance of the large stellate cells in lamina IV*b*, and a general reduction of cells in all the laminae. ×72.

Fig. 7. Section of the normal visual cortex of O.M. 149, stained with Bodian's silver method. The section shows the granular and infragranular laminae. Note the characteristic large cells of Meynert in lamina VI. ×90.

Fig. 8. Corresponding section through the isolated cortex of O.M. 149. Note the shrinkage and denser staining of the Meynert cells which occurs 14 days after isolation. ×90.





LE GROS CLARK AND SUNDERLAND—STRUCTURAL CHANGES IN THE ISOLATED VISUAL CORTEX

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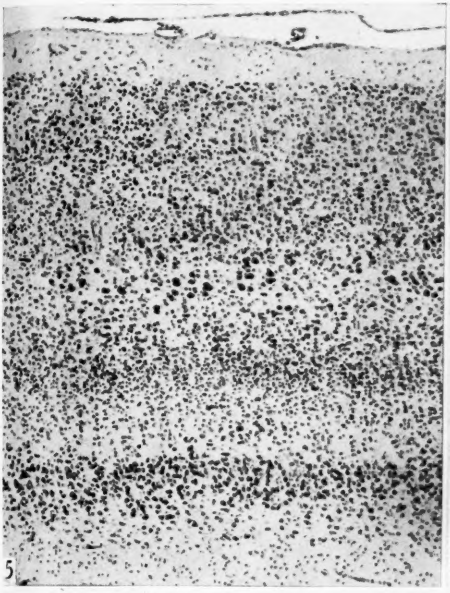

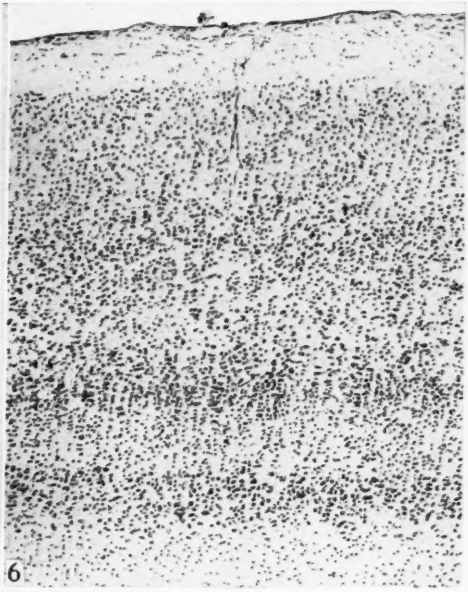

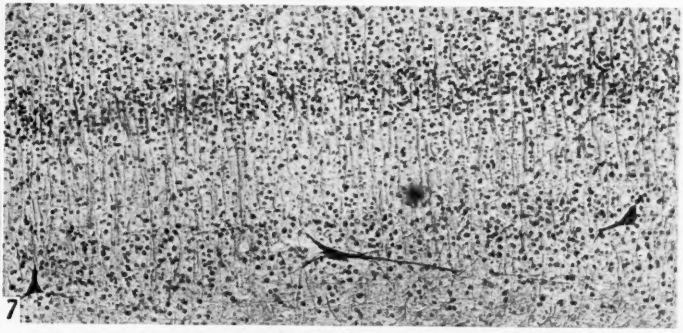

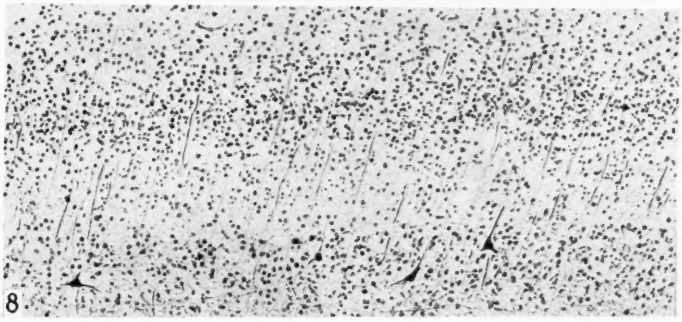

LE GROS CLARK AND SUNDERLAND—STRUCTURAL CHANGES IN THE ISOLATED VISUAL CORTEX

UNIV. OF MICH.

THE RELATION OF FINGER-NAIL GROWTH TO NUTRITIONAL STATUS

By MARY L. GILCHRIST
Assistant Medical Officer, Borough of Leytonstone

AND THE LATE L. H. DUDLEY BUXTON
Department of Human Anatomy, University of Oxford

In 1937 Le Gros Clark & Buxton made a series of observations on the nail growth of Oxford undergraduates and children from private and elementary schools in Oxford. The series which they examined were intended to serve as a basis for future work and we have followed the technique outlined in their paper. This technique is as follows:

With a small file (of the type employed for opening glass ampoules), a fine transverse scratch was made across the nail at a distance of about 2 mm. from the margin of the lunula. The distance between the proximal margin of the file-mark in the median axis of the nail and the central point of the margin of the lunula was then measured with a Beck's Luminex Magnifier fitted with a micrometer scale. With this instrument it was usually possible to take measurements to within 0·1 mm. After an interval of approximately one month, a second observation was made, in this way recording the displacement distally of the file-mark during this period. The daily growth of the nail could then be calculated. With regard to this technique it may be emphasized that the margin of the lunula provides a fixed base-line, for it marks the edge of the germinal matrix of the nail-bed, which shows quite an abrupt structural transition into the sterile matrix. In the case of the thumb, and the index and middle fingers, the margin is usually sharp enough to allow of accurate measurement without difficulty. In the ring and little fingers the outline is not always so distinct, and considerable care may be required to obtain reliable measurements. In some individuals the lunula may hardly be visible at all in these fingers, and in such cases it was not possible to make an observation. In cases where the margin of the lunula is obscured by a forward extension of the eponychium, it can often be made more apparent by smearing a trace of vaseline over the surface of the nail. The displacement of the file-mark in the course of a month amounted usually to about 3 mm. In some instances of exceptionally rapid growth (e.g. in nail-biters), the mark was found to have disappeared at the free edge of the nail by this time.

In the previous paper it was found that there was no significant difference between right- and left-hand growth, between male and female, or between age groups. Our observations, as will be seen later, confirm this. The authors

also found that there was no significant difference between the growth of the finger nails of undergraduates and of children attending both private and elementary schools.

The individual observations suggested that a more detailed examination of the effect of nutritional status on nail growth should be undertaken, and the results of such an enquiry are embodied in the present paper. It should be stated at the outset that, as the seasonal variation is very considerable, our observations, which were taken in the autumn and winter, are not absolutely comparable with the Oxford figures taken in the spring and summer.

ASSESSMENT OF NUTRITIONAL STATUS

The assessment of the nutrition of the children who are the subject of this investigation was along the lines of the clinical examination which has been the subject of so much criticism lately, and which of itself cannot bear the scrutiny of statistical examination. There is, however, a factor in this investigation which may have eliminated in part the subjective errors which do occur. The children were drawn from three different types of school in the borough of Leytonstone in East London, a technical school, two central schools, and two elementary schools. The elementary schools drew their children from the poorest wards in the district, and as the school staffs were asked to pick out the children whom they knew were from homes where privation was the rule, the task of the assessing officer was, in consequence, less liable to error. Out of the ninety-five elementary school boys who completed the test, seventy-nine were assessed as of C nutrition[1], so that there was substantial agreement between the assessment and what was the probable economic condition of the homes of the children.

The remainder of the A, B, and C nutrition groups attended the central and technical schools. This group of children had been subjected to a routine physical examination a few weeks previously to these tests being made, either by M. L. G. or by another school medical officer, who from previous experience is known to have a very similar standard. The children were reassessed again quickly, and if found not to agree with the previous assessment, the second one was adhered to so that the same standard might prevail throughout. It was found necessary in only nine cases (out of 284) to change the assessment. It cannot be claimed that the three groups do not overlap in any way, since we do not claim infallibility for our clinical acumen where so many have been proved inaccurate before; but with the rough economic test which was applied to the majority of the C group, it seems justifiable to say that that group at least was likely to contain few A's or B's.

[1] The letters A, B, and C denote the grades of nutrition as laid down by the Board of Education for the guidance of school medical officers when making the returns of the state of nutrition in their area. A means excellent, B means normal, and C means subnormal nutrition.

METHOD OF COLLATING OBSERVATIONS

The observations were first reduced to a daily average growth, since it was not possible always to allow the same interval of time between measurements. They were then divided into the following categories within the nutrition groups A, B, and C; normal (that is, those who showed no evidence of nail-biting), nail-biters, and total observations. The sexes were counted separately but subsequently pooled. Le Gros Clark & Buxton, in the paper already referred to, showed that on their data there was a marked distinction between nail-biters and normal, but no significant difference between the sexes. They also found a high correlation between right and left hands. It was considered therefore that one hand would provide a convenient check on the other. The means and standard deviations were then calculated and will be found in Table I. Table Ia gives the numbers on which the various constants are based. The discussion which follows will be most easily understood if the table is read downwards.

Table I. *Average daily growth in micromillimetres*

		Right hand			Left hand		
	Class	Normal	Biters	Total	Normal	Biters	Total
				Means			
♂	A	107·21	119·00	110·35	107·86	121·70	111·35
	B	107·12	119·49	109·62	107·23	119·17	109·75
	C	99·71	109·98	100·96	100·69	109·21	102·44
♀	A	106·17	119·47	108·83	105·86	120·17	109·50
	B	104·45	122·10	107·85	100·26	124·10	104·95
	C	101·00	121·00	106·00	94·22	110·33	98·17
♂+♀	A	106·22	119·42	109·98	107·39	121·34	110·25
	B	106·27	119·90	109·07	104·02	120·60	108·21
	C	98·92	111·33	101·54	100·10	109·50	102·10
				Standard deviations			
♂	A	14·19	—	15·72	15·13	—	16·65
	B	16·84	—	18·69	16·10	—	18·42
	C	16·54	—	16·77	20·83	—	20·07
♀	A	20·56	—	20·71	14·29	—	17·42
	B	16·81	—	19·94	19·11	—	22·35
	C	—	—	—	—	—	—
♂+♀	A	15·87	16·59	16·96	14·85	17·82	16·77
	B	16·47	22·69	19·17	17·35	23·78	19·79
	C	15·68	16·70	17·08	19·64	16·00	19·41

Table Ia. *Number of cases in each group*

	Normal		Biters		Total	
Class	Male	Female	Male	Female	Male	Female
A	55	18	20	6	75	24
B	90	42	24	10	114	52
C	81	9	21	3	102	12

First, a superficial examination shows that the male means for groups A and B are consistent, and it needs no statistical treatment to affirm their similarity.

Group C appears to be different, and accordingly this difference was reserved for exact statistical treatment. The female means show a gradual diminution from group A to group C, but as the numbers in groups A and C were so few, this might be the result of random sampling. An examination of the "biters" and the totals confirmed the result of the first examination, except that the female nail-biters showed the only actual inconsistencies in the table. However, an examination of the numbers on which they were based amply accounted for this. Both sexes were then pooled. Definitely consistent results were obtained, and the similarity between A and B once more appeared, except that the left hand alone suggested that possibly there was a gradation between the groups even there.

The standard deviations are singularly liable to be affected by random sampling, but an examination of the right hand figures showed a surprisingly close approximation, and there was certainly nothing like the contrast between A and B on the one hand, and C on the other, which appeared in the means. The greatest difference which occurs is between A and C on the left hands of the males. This may be roughly tested as follows. The standard error of the standard deviation is equal to the standard deviation divided by the square root of twice the number of cases. The standard error then of the standard deviation of the males of C group is approximately $2\ (20+\sqrt{(102)})$, but the difference between 20·07 and 16·65 is 3·32, which is less than twice the standard error of the former figure alone, and the difference may reasonably be set down to random sampling. The point is discussed later.

RELATION OF NUTRITIONAL STATUS AND NAIL GROWTH

Having from a superficial examination concluded that A and B groups were similar in the rate of nail growth, it was decided to apply more exact treatment to possible differences between these and C. But since, if there was a difference between B and C, *a fortiori*, there would be a difference between C and A, only the former case has been considered.

A comparison between two means raises certain difficult points in regard to method. Since the standard deviations are so similar, it seemed best to use the following test:

$$\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\left(\frac{\Sigma_1-\Sigma_2}{n_1+n_2-2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)\right)}},$$

where $\bar{x}_1$ and $\bar{x}_2$ indicate the means compared, Σ the sums of the squares ($S\,(x-\bar{x})^2$ in Fisher's nomenclature), and n_1, n_2 the number of observations in each sample.[1]

The disadvantage of tests between means is, first, that we cannot tell from the test whether the difference, if it appears, is due to a real difference in means

[1] For a discussion on the use of this formula, see *Biometrika*, vol. XXIX, p. 350 (1938).

or to a difference in the variability. It has already been pointed out that the standard deviations are very similar so that this difficulty appears to vanish. The second difficulty, which is inherent in all statistical method, is that the number of cases as well as the measurements come into the formula, and therefore where numbers are few we may not realize the existence of a real difference, while similarly where numbers are great we are possibly inclined to give weight to rather too fine differences. In this particular case, when the sexes were analysed, a difference was found between B and C in the males, but not in the females. This was almost certainly due to the smaller number of females. It was decided therefore, first of all, to take the pooled sexes, and to use the formula already given. The results were as follows:

Comparison	Degrees of freedom	t	
		Right	Left
A and B:			
Normal	203	0·0148	0·985
Total	263	0·734	1·185
B and C:			
Normal	220	3·321	3·397
Total	278	3·366	2·597

Since from Fisher's table, $p=0{\cdot}01$, $t=2{\cdot}750$ with 30 degrees of freedom, $t=2{\cdot}57582$ with ∞ degrees of freedom, we may reasonably conclude that there is no difference between the means of A and B, but there is between those of B and C, thus confirming our decision based on superficial examination. In the case of the biters, the figures for the right hand are 0·0879 between A and B and 1·52 between B and C. This latter figure is not significant, but the difference, if such exists, may be obscured partly by the great variation of the biters and partly by the small number of cases. Applying a similar method to the males alone on the right hand, t for B and C normal is 3·3197, which is certainly significant, and 3·5948 for the total B and C. For the total on the left hand it is 2·7992. On the other hand, for the normal on the left hand the figure falls to 2·331. Even for the normal females, of whom there are only 9 in the C group, the value of t is 2·175, which is equivalent to a p of less than 0·05. This is of doubtful significance, but the doubt is probably due to the lack of numbers rather than to any real lack of difference between the two groups.

Turning to the differences between the standard deviations, we have used Fisher's z test, where z is equal to the difference between the natural logarithms of the two standard deviations compared. Tests were made between the various A and B groups and between the B and C groups. Only one significant difference was found, between the male B and C normal, where $z=0{\cdot}3575\pm0{\cdot}0966$. It will be remembered that there was an irregularity here also in the means. It should, however, be noted that the differences on the right hand between A and B, although not significant, are approaching significance. No conclusion can be reached one way or the other from the present evidence, but possibly further evidence may show that the A group really are, as they appear super-

ficially to be, less variable than the rest. One of us in examining a previous series noticed that in the best nourished persons extreme variations appeared to be less common.

This important point leads to the relation of these averages to the individual. It is clear that we cannot assess a single person by his nail growth. The groups overlap very considerably, as is shown by the graph. It is only when comparatively large numbers are studied that the differences appear.[1]

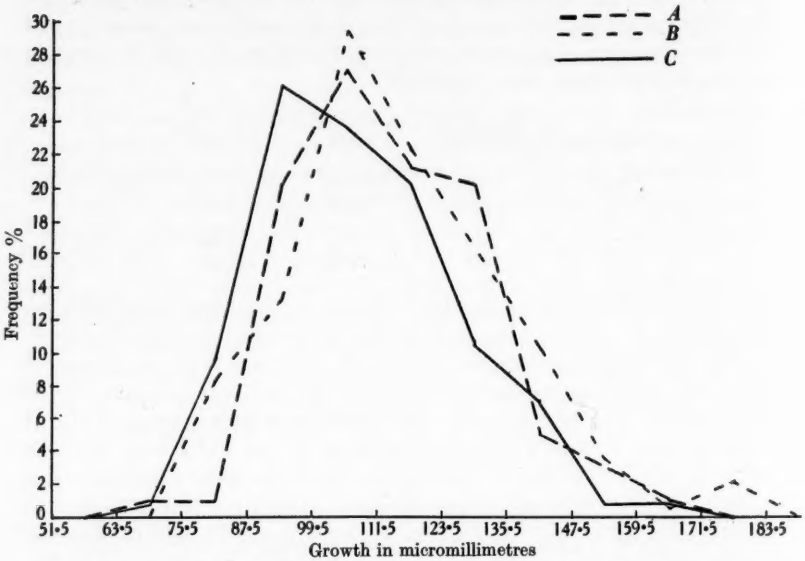

Fig. 1. Shows the percentage frequency of right hand nail growth in the three nutritional groups. The figure is based on all the measurements, and, for this purpose, both sexes and nail-biters and normal children have been included. The absolute data, not reduced to percentages on which the figure is based, are shown in Table II.

A friendly critic has suggested, that in view of what is written above, the analysis of variance would be a more efficient method of approach. The choice of method would appear to rest very largely on the value of the clinical assessment of A and B groups. Using the analysis of variance for right hand normal growth, combining males and females, one gets the following results:

	Sums of squares	Degrees of freedom	Mean variance	Log_e
Between groups	3587·9	2	1743·9	7·4640
Within groups	77689·6	292	266·1	5·5839
			Difference	1·8801

$z = 0{\cdot}9400$

As the value with 2 degrees of freedom and ∞ for the 1% point of z is 0·7636, one need not doubt that the difference in growth between children of different nutritive status is real. The value of z for the left hand normal is 0·6317, which is less than the 1% point but greater than the 5% point. For the nail-biters, the value of z for right and left hand respectively is 0·2055 and 0·3290, neither of which, as in the t test, are significant. These results form an interesting corollary to those which were obtained by the t test.

Table II. *Summarized distribution of average daily growth in micromillimetres of right hands*

A

Midpoint of group	Male		Female		Total
	Normal	Biters	Normal	Biters	
171·5	—	—	—	—	—
159·5	—	1	—	—	1
147·5	1	—	1	1	3
135·5	1	2	1	1	5
123·5	8	9	3	—	20
111·5	13	1	3	4	21
99·5	18	5	3	1	27
87·5	13	1	6	—	20
75·5	—	—	1	—	1
63·5	1	—	—	—	1
Total	55	19	18	7	99

B

Midpoint of group	Male		Female		Total
	Normal	Biters	Normal	Biters	
171·5	—	1	—	1	2
159·5	—	1	—	—	1
147·5	2	2	—	2	6
135·5	8	2	5	2	17
123·5	5	7	6	—	18
111·5	24	5	7	1	37
99·5	29	3	14	3	49
87·5	12	3	6	1	22
75·5	10	—	4	—	14
63·5	—	—	—	—	—
Total	90	24	42	10	166

C

Midpoint of group	Male		Female		Total
	Normal	Biters	Normal	Biters	
171·5	—	—	—	—	—
159·5	1	—	—	—	1
147·5	1	—	—	—	1
135·5	3	3	1	1	8
123·5	6	4	1	1	12
111·5	16	5	2	—	23
99·5	19	5	2	1	27
87·5	24	4	2	—	30
75·5	10	—	1	—	11
63·5	1	—	—	—	1
Total	81	21	9	3	114

SUMMARY

1. The nail growth was examined of a number of children from elementary schools in Leytonstone, East London, who had been independently graded into three groups, A, B and C, according to their nutritional status.

2. The correlation between nail growth and nutritional status has been listed.

3. Considerable individual variation in nail growth was found, but examination of the group means showed that while there was no difference in the rate of nail growth between groups A and B, the nails of poorly nourished children in group C grew more slowly.

4. The evidence suggests that the nail growth of well-nourished children shows less variation than that of the more poorly nourished.

Our thanks are due to Prof. Le Gros Clark for suggesting this line of research and for his assistance, to Dr A. W. Forrest for permission to carry out the work, and to the Head Masters of Leyton Senior Schools, without whose co-operation the data could not have been collected.

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THE ANTERIOR SUPERIOR ALVEOLAR NERVE AND VESSELS

By FREDERIC WOOD JONES
University of Manchester

It has always been a stumbling block for the student of anatomy that, while only posterior and anterior superior dental vessels are described, there are three groups of superior dental nerves—posterior, middle and anterior—defined in every text-book. The posterior and anterior arteries and nerves are described as running in company: but the middle superior nerves are apparently unaccompanied by vessels. Moreover, it is apparent that the course of none of these nerves is given in any text-book with sufficient precision to satisfy the demands of modern surgery. Although the present investigation is concerned mainly with the anterior vessels and nerves, it is necessary to clarify the position by defining the other members of the series.

THE POSTERIOR SUPERIOR DENTAL NERVES

The nervi alveolares superiores posteriores arise from the nervus infraorbitalis just before it enters the posterior end of its bony canal. As a rule, the branches are three or occasionally four in number, and not all of them are destined for the supply of the teeth, for, as a rule, one branch at least fails to enter any of the bony foramina on the posterior surface of the maxilla. This branch has been distinguished as the nervus buccalis by many authors, and by Rudinger was designated as the ramus maxillaris externus. It is the branch that runs to the "gum and adjacent part of the mucous membrane of the cheek" (Quain, 1897), and which may at times replace the buccinator nerve itself (Turner, 1867). By Charles Bell (1803), this nerve was considered as normally supplying the buccinator muscle, for he says, "it takes a course on the outside of the maxillary bone, and supplies the gums and alveoli and buccinator muscle". Ellis (1840), Wilson (1840), Cruveilhier (1842), the Ledwiches (1852) and many other authorities have also described the buccinator branch going to the muscle, and Cloquet (1828) describes, in addition, a branch to the pterygoideus internus. The nerve seems to be of sufficient importance to be designated by a special name, and that adopted by Rudinger appears the least ambiguous. The dental branches proper of the posterior nerve are usually derived from two main branches. These branches were at one time named in descriptive anatomy, the superior and inferior branches of the posterior dental nerve (Ellis, 1840; Cruveilhier, 1842; Ledwich, 1852; etc.). The superior branch, by far the smaller of the two, tunnels the lateral wall of the maxillary antrum by running at the level of the malar tuberosity, the larger inferior branch being well below the

tuberosity and running a course below and parallel to the transverse facial part of the canal for the anterior nerve. The canal for the inferior dental branch in the posterior and lateral walls of the antrum of Highmore may be readily followed as far forwards as the root of the canine tooth.

The middle superior dental nerve is also known as the ramus dentalis superior minor of Beck, and as the nervus alveolaris superior anterior minor, and has a somewhat remarkable history in anatomical literature. So far as I am able to ascertain, the first mention of such a nerve is in the *Anatomist's Vade Mecum* of Erasmus Wilson, first published in 1840. The description of the nerve given in this and in all subsequent editions of the work is, however, extremely meagre. Indeed, it is almost beyond question that the middle dental nerve of Wilson is the same thing as the superior branch of the posterior nerve of Cloquet *et al.* The *Vade Mecum* was translated into German, appearing in 1845 as the *Compendium* of Hollstein. The nerve became stereotyped in German descriptive anatomy, and is described and figured (erroneously) by Henle (1868). Apart from Wilson's popular little anatomical treatise, English text-books did not recognize the existence of this nerve until a considerably later period, and in France it was ignored by Testut even so late as 1905. No mention is made of it in the eighth edition of Quain (1876), but it appears in the ninth (1882). Here it is defined as follows: "It leaves the superior maxillary in the hinder part of the infraorbital canal, and is directed downwards and forwards in a special canal in the outer wall of the antrum to the bicuspid teeth." It is mentioned that the nerve is inconstant and not uncommonly "conjoined with the anterior". For the past half century, it has uniformly taken its place in English descriptive anatomy without, apparently, anything having been added to the earlier accounts. The story of its advent into the more recent English and French text-book literature is a curious one. The beautiful anatomical plates of J. B. Léveillé appeared in 1850 (second, definitive, edition, 1866), and their influence on descriptive anatomy still continues in their innumerable reproductions and modifications in so many of the text-books published to-day. In Pl. 27, figs. 1 and 5, the posterior superior alveolar nerve is very accurately shown in three divisions upon the outer surface of the posterior part of the maxilla. All three branches are indicated in the figures as being part of the posterior nerve and, in Hirschfield's text accompanying the plates, no mention is made of a middle superior alveolar nerve. It is a remarkable fact that, when descriptions of a middle nerve became current, the English text-books for the most part (see Quain, 1897, *et al.*) placed an index line on the most anterior of the three branches to signify the "middle" nerve described in the text, while the French (see Cunéo, Poirier (1901), ed. II, *et al.*) selected the intermediate branch. It is still more remarkable that Cunéo, in reproducing Léveillé's Pl. 27, fig. 5, selected the intermediate branch, while, in reproducing his Fig. 1, he indicated the anterior one as being the "middle" nerve. Sappey (1877), in reproducing Léveillé's Pl. 27, fig. 5, selected the anterior branch as the "middle" nerve; but in reproducing

Fig. 1, included this branch in the posterior nerve and gave no indication of a middle. Another fact of importance in this connexion is that while, according to the descriptions in the text, the middle superior alveolar nerve arises from the superior maxillary in the infraorbital canal, it is obvious that neither of the nerves so named in the plates arises from the parent trunk after it has entered the canal. This point is also particularly well shown in Spalteholz's (1901) Fig. 765. With the exception of Henle's obviously inaccurate Fig. 240, I have been unable to discover any illustration of a middle superior alveolar nerve arising in the infraorbital canal (as described in the accompanying text) and I am inclined to agree with Robert Knox (1853), when he says of the anterior nerve: "This is the only branch which the superior maxillary nerve sends off whilst passing through the suborbitar canal." Cruveilhier (1842), and many other anatomists of the period, had previously insisted that the anterior dental nerve was the only branch given off in the canal. I have, so far, been unable to identify a classical middle superior dental nerve in dissections or to trace the canal, in which it is said to run, in dry bones. Its occurrence must, I think, be a matter of some infrequence, and it is to be doubted if its retention in normal descriptive anatomy is altogether desirable.

Anterior superior dental nerve, also known as nervus alveolaris superior anterior major, and as nervus naso-dentalis. The B.N.A. designation—rami alveolares superiores anteriores—is not to be commended, since it tends to obscure the fact that the nerve is a single large trunk for the greater part of its course, and it also perpetuates and accentuates the errors of most recent descriptions. The common description, that the nerve consists of a series of branches running in minute bony canals in the anterior wall of the antrum, stands in need of correction. The modern descriptive accounts of this nerve are extremely meagre. It is often described as "a small branch" (Morris, 1893, *et al.*). It is, however, a nerve of considerable size, being by far the largest branch given off by the trunk of the nervus infraorbitalis. Knox (1853) has said: "it is so large a branch that it might almost be viewed as a bifurcation of the great terminal branch of the superior maxillary", and Cruveilhier (1842) employed exactly the same descriptive terms. This is, perhaps, somewhat of an over-estimate, but it may be accepted that, as a general rule, the nerve is at least rather more than one-third the size of the parent trunk. Its point of origin is usually not defined further than the somewhat vague statement that it arises "near the infraorbital foramen". Cunéo is more precise and places its origin 5 or 6 mm. behind the infraorbital foramen. Macalister (1889) gives the anterior third of the infraorbital canal as its site of origin, and Ellis (1840) says: "it quits the main nerve while in the infraorbital canal and about one inch before this appears on the face". I find that its origin from the parent trunk is generally situated posterior to the mid-point of the canal and to be, on an average, some 15 mm. or more behind the infraorbital foramen (Fig. 1). It is, indeed, by no means uncommon to find the nerve separate from the infraorbital trunk at the extreme hind end of the canal. As to the aspect of the infraorbital

nerve from which it takes its origin, no statement is to be found, so far as I am aware, in any current text-book. It is sometimes figured (Fort, 1887, Fig. 470; Morris, 1893, Fig. 446, *et al.*) as arising from the medial side of the main nerve and continuing, medial to the infraorbital foramen, to the incisor teeth. More often, it is depicted as arising from its lower aspect. All figures in current works that are based on Léveillé's plates, show the nerve correctly as arising from the lateral aspect though, with very few exceptions, attention is not called to this fact in the text. As a matter of fact, the nerve always arises from the lateral aspect of its parent trunk, and, as soon as it is free from the main sheath, runs laterally in a bony canal of its own. This bony canal on the orbital floor is usually some 10 mm. or more in length and nearly 2 mm. in diameter. It runs in a lateral direction and reaches the anterior margin of the orbit as much as 7 or 8 mm. lateral to the lateral margin of the infraorbital foramen. The canal

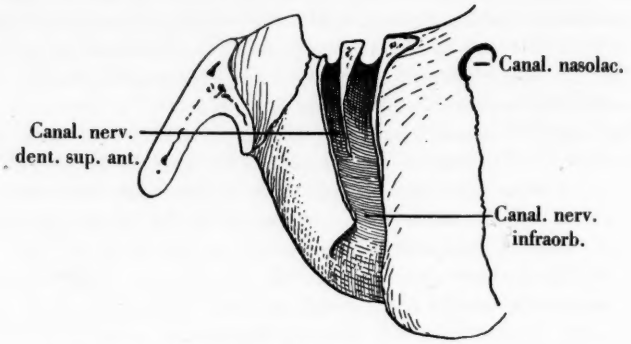

Fig. 1. The floor of the left orbit to show the separate canal for the nervus alveolaris sup. ant.

then turns downwards in the anterior wall of the antrum (Fig. 2). This turning point may be marked on the anterior surface of the maxillary bone at a spot some 6 mm. below the inferior orbital margin, or just below the malar-maxillary suture, 1 cm. lateral to the mid-point of the infraorbital foramen. As the canal runs downwards in the anterior wall of the antrum, it frequently forms a prominent ridge or buttress on the inner aspect of the upper part of the antrum, between which and the buttress often formed by the infraorbital canal itself, there is commonly a deep recess. The downward course is, however, not continued far, for directly it has fallen some 3 or 4 mm. below the level of the infraorbital foramen, the canal turns mediad, passing below the infra-orbital foramen in a gentle curve and rising again as it passes towards the narial margin. This is the transverse facial part of its course (Fig. 3). The average length of this portion of the canal is slightly over 2 cm. and it is to be noted that whilst the lateral three-fourths lie in the anterior wall of the antrum, the medial fourth lies in the lateral wall of the nasal chamber, medial to the antral

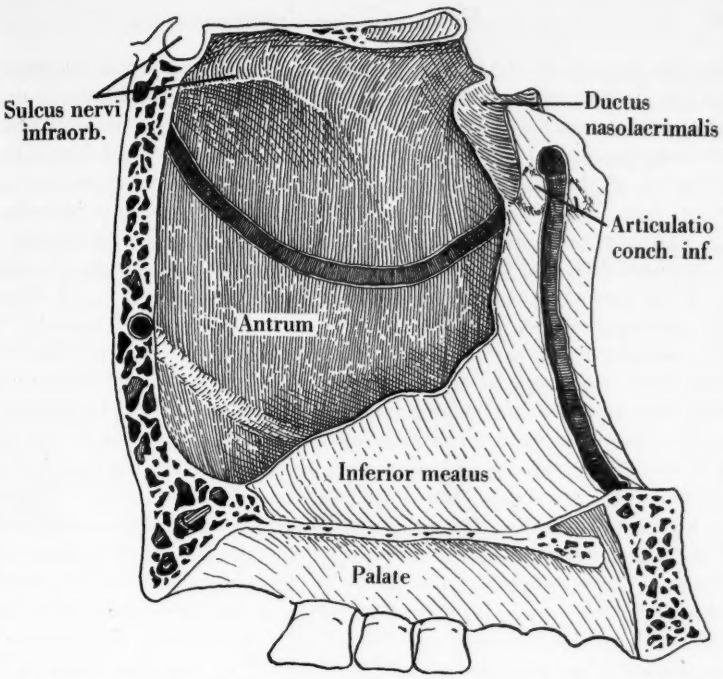

Fig. 2. The canalis nervi alveolaris sup. ant. displayed by removing its posterior wall.

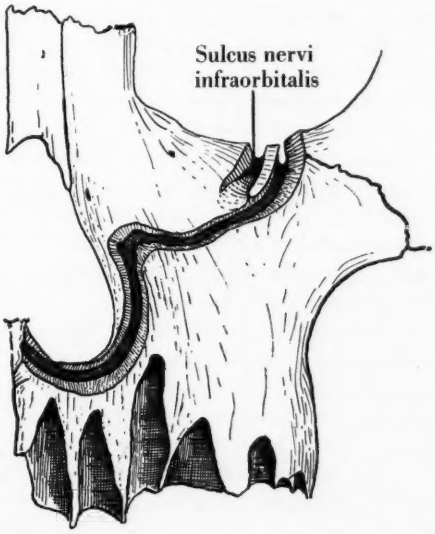

Fig. 3. The canalis nervi alveolaris sup. ant. displayed by removing its anterior wall.

cavity (see Fig. 5). At the medial extremity of its transverse facial course, the canal rises in very characteristic fashion, so that its outer and inner ends are almost on the same level. This rise at the medial end brings the canal and its contained vessels and nerves very constantly to the level of the anterior attachment of the inferior turbinate bone and the lower end of the naso-lacrimal duct. At this point, the canal is somewhat dilated, and branches of nerve and vessels pass inwards to the nasal chamber to the concha inferior, the naso-lacrimal duct and the meatus inferior. Communicating with the dilatation of the medial end of the transverse facial portion of the canal, there is often a foramen which issues on the lateral side of the facial aspect of the narial margin, about half way, or even considerably higher, up the narial orifice. This canal appears to be entirely vascular, no branch of the nerve having been traced to it. Having arrived at this point on the lateral wall of the nose, overlying the anterior attachment of the concha inferior, the canal now turns abruptly downwards, tunnelling the bone around the margin of the narial opening, some 3 mm. from the free edge of the maxilla constituting the bony margin of the orifice. The canal here follows the curve of the narial margin and is situated between the medial aspect of the alveolus for the canine tooth, with the upper extremities of the alveoli of the incisors, and the nasal cavity. In this part of its course, it gives off its first conspicuous dental branch to the canine, and smaller branches may be traced to the incisors. Meanwhile, the lower branch of the posterior superior alveolar nerve, which has run round the wall of the antrum at a lower level (some 8 or 9 mm. below the transverse facial canal of the anterior superior alveolar), can be traced to the anterior limits of the antral wall, or to the back of the canine alveolus. Having descended along the narial margin to the floor of the nasal chamber, the canal now occupies a position that was, I believe, first described by Macalister, but (with the exception of a later paper by Burkitt and Lightoller) subsequently overlooked by most authors. In describing the inner margin of the narial aperture, or "paraseptal ridge", Macalister (1898) wrote: "it is lost posteriorly on a slightly convex eminence, which descends from the outer wall of the nasal cavity, downwards and inwards to the front of the anterior palatine foramen: this eminence is hollow, and contains in it the anterior superior dental nerve and its accompanying vessels". Burkitt & Lightoller (1923), say of this same ridge that "it runs to the lateral wall of the nose, near the inferior turbinate at its anterior end. It is often seen to be channelled and to contain the anterior superior alveolar nerves and vessels to the incisor teeth." This paraseptal or dental ridge is, as a matter of fact, simply the continuation of the canal for the anterior superior alveolar nerve and vessels, and in it the canal is continued to the septum of the nose just anterior to the anterior palatine foramen (Fig. 4). At the septum, the canal opens somewhat variably by a foramen that is often conspicuous in the dried skull, but of which I can find no description in the literature. Through the foramen passes the terminal branch of the anterior superior dental nerve which Luschka (1867) designated the ramus nasalis.

Through this foramen also issues the terminal branch of the anterior superior alveolar artery, which enters into, and makes a considerable contribution towards, the anastomosis of the tache vasculaire de la cloison: a vein from this region of the septum also enters the foramen. The whole course of the bony canal for the anterior superior vessels and nerve is some 55 mm. in length. Of this total length, some 15 mm. traverse the orbital floor, some 20 mm. are occupied in the curved transverse facial part of its course, and 20 mm. in the curved circumnarial course (Fig. 5).

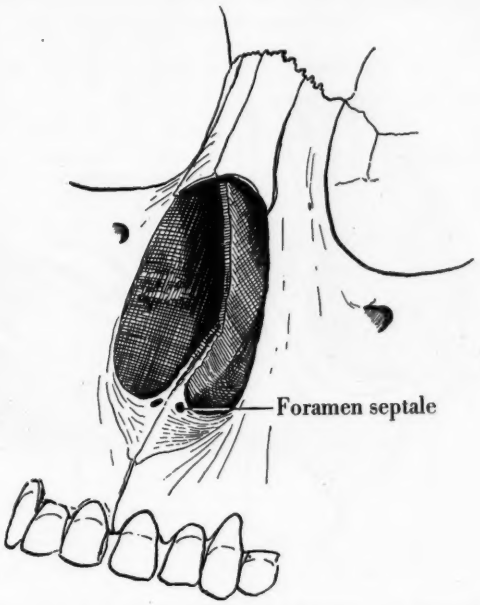

Fig. 4. The narial margin to show the parasepta ridge and foramen septale.

It is quite obvious that about a century ago there were many excellent descriptions of certain parts of this canal current in text-book literature. In 1840, Ellis wrote: "the anterior dental nerve may be traced for some distance in its canal in the bone." Cruveilhier in 1842 gave a good account of the first part of its course, and the Ledwiches in 1852 only failed to trace it in its circumnarial portion, for they say that "having arrived at the root of the nasal process, it divides into a number of branches". To Macalister (1898) belongs the credit of describing (incidentally) the terminal part of the canal. Modern anatomical description of the course of this nerve, even if it is attempted at all, appears to be considerably less precise and less accurate than that available to students a hundred years ago. Heath's *Practical Anatomy* was a widely

used dissecting manual, and in it the student is told that "the branches of the dental nerves can only be traced to the teeth by removing the outer plate of the alveolus, but the expenditure of time and trouble necessary for this will not be repaid by results". It may be that anatomists of a later generation accepted Heath's advice, and the fact that the nerve ran in a wide canal, and not in a series of minute passages, was lost sight of. Since this long canal is so conspicuous a feature of the maxillary bone and is often sharply defined in skiagrams, it would seem useful to distinguish it by some defined anatomical term. It might be suggested that, owing to its double curved course, the term

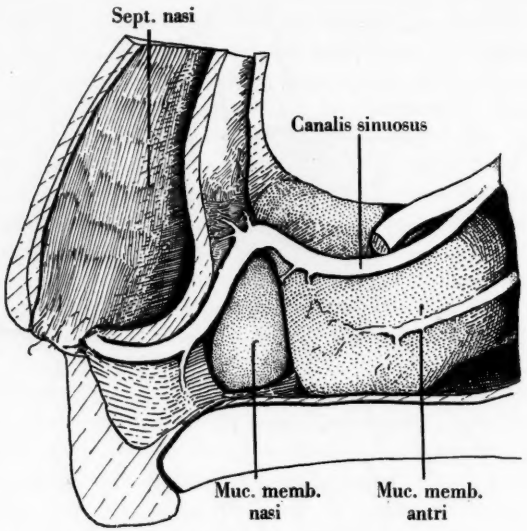

Fig. 5. Specimen prepared by decalcifying and removing the whole of the facial portion of the maxilla in order to display the canalis sinuosus for the ant. sup. alveolar nerve and vessels.

"canalis sinuosus" would be appropriate; while the terminal foramen for the exit of the septal (Luschka's ramus nasalis) nerve and vessels might be defined as "foramen septale".

In the surgery of the antrum, it is important to realize the large size and the constancy of this canal as a corrective to the common impression that the anterior superior alveolar nerve consists of a series of small branches running in small bony canals down the front wall of the antrum. It is also of importance to realize how considerable a contribution the anterior superior alveolar vessels and nerves make to the supply of both the septal and lateral walls of the nasal chambers. The septal branches, especially of the veins and arteries, are probably of far more than mere academic interest.

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A NOTE ON THE COMPARATIVE SIZE OF THE COCHLEAR CANAL IN MAMMALS

By J. A. KEEN
Department of Anatomy, St Thomas's Hospital Medical School

The late Dr Albert Gray's *Labyrinth of Animals* published in 1907 remains the standard work on the labyrinth of mammals, birds, reptiles and amphibians. The two volumes contain stereoscopic photographs of the labyrinths of seventy-five species of animals with tables of measurements. The main

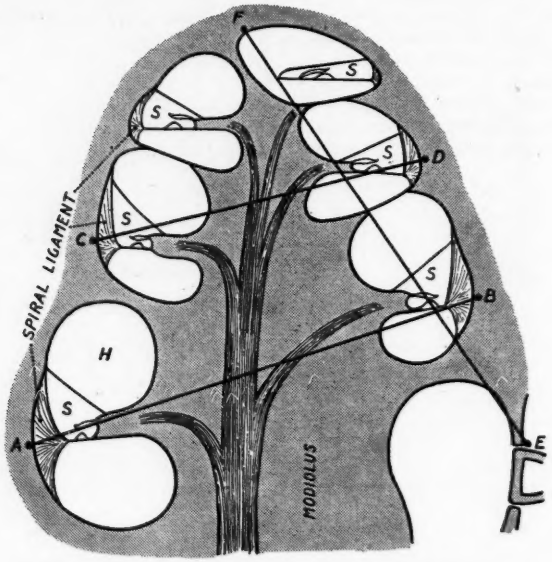

Fig. 1. Section of a typical mammalian cochlea with three turns. (Diagrammatic.) *S* = scala media.

impression which one obtains from the descriptions and dimensions given is one of great diversity of both the cochlear and vestibular parts.

The fully developed cochlea is only found in mammals, a spirally coiled tube being characteristic of all orders. The number of turns in the spiral varies from $1\frac{1}{2}$, e.g. in the mouse, to $4\frac{1}{2}$, e.g. in the guinea-pig, the human cochlea occupying an intermediate position with $2\frac{3}{4}$ turns. The spirally coiled scala media or ductus cochlearis (*s*) together with the spiral ligament lies against the outer endosteum of the spiral bony canal (Fig. 1). In the preparations of

the membranous labyrinth, made by Gray, the coiled scala media was separated out, together with a bridge of tissue, composed mainly of nerve fibres, which passes across the osseous spiral lamina and the modiolus; the bony parts were removed by decalcification. Gray's measurements, which are set out in Table II, show the distances from points *A–B*, *C–D* and *E–F* (Fig. 1). It will be obvious that these measurements actually represent dimensions of the bony cochlea.

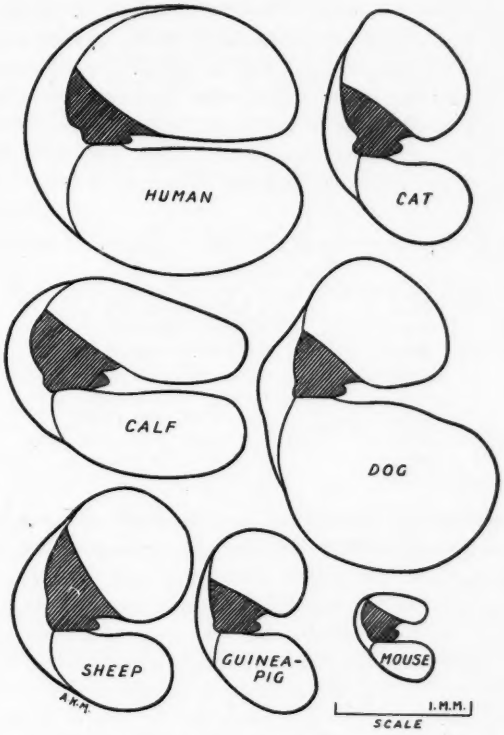

Fig. 2. Appearance of basal coil of cochlea of various mammals in cross-section. Tracings from microphotographs taken at the same magnification.

When the dimensions of the scala media are studied in microscopic sections of the cochlea, there is found to be far closer similarity in different mammals than might be expected from Gray's measurements. The whole bony cochlea may indeed be relatively large or small. It may be a flattened out structure with wide separation of the scala media in each whorl, or a sharp-pointed organ with a steeply ascending spiral and close approximation of the whorls. The bony canals in cross sections may show as wider or as smaller spaces. Yet,

in spite of all these differences, there are found very close resemblances in the scala media, and more particularly in the width of the basilar membrane.

The following mammalian cochleae were available for study: man, cat, sheep, calf, dog, guinea-pig and mouse. In order to make measurements which are comparable it is necessary to section the cochleae in corresponding planes. The most useful plane is one in which the whole of the modiolus and every coil is seen. As is well known, the width of the basilar membrane gradually increases from the base to the apical turn, and there was no exception to this rule in the seven types of cochleae which were examined. In each section one of the basal coils was chosen for comparison. It was decided to select the one further away from the vestibule, i.e. coil *H* in Fig. 1, for the reason that the coil on the vestibular side often showed some feature peculiar to the species, e.g. a bulging out of the floor of the scala tympani. The coils chosen were photographed at the same magnification. Tracings were then made from the microphotographs, the scala media being shown as a shaded area (Fig. 2).

Table I. *Measurements of the width of the basilar membrane (in millimetres)*

	Base	Intermediate coils						Apex
Human	0·20		0·23	0·28	0·37			0·48
Cat	0·20	0·22	0·25	0·28	0·33			0·37
Sheep	0·20		0·25	0·32				0·35
Calf	0·22		0·23	0·28	0·32			0·35
Dog	0·27		0·28	0·32	0·33	0·33		0·40
Guinea-pig	0·15	0·20	0·21	0·22	0·23	0·25	0·25	0·25
Mouse	0·13			0·15				0·17

The shaded parts in the illustrations are the areas enclosed by the basilar membrane below, the vestibular (Reissner's) membrane above, and the spiral ligament on the outside. The sulcus spiralis internus, the organ of Corti, hair cells, supporting cells and the membrana tectoria are enclosed in the darkened area. The limbus laminae spiralis and the stria vascularis have not been included since there is some doubt whether these latter structures are developed from the original epithelial lining of the otic vesicle or not.

Measurements of the basilar membrane in the coils from base to apex are found in Table I. It has been recommended that the basilar membrane should be measured from the tip of the spiral ligament to a point corresponding to the base of the inner rod of Corti (Bethe, 1926) and this method has been followed. Determination of the width of the basilar membrane in this way has the advantage that the free part of the membrane is measured. The base of the inner rod of Corti usually rests on the extreme edge of the osseous spiral lamina or very near it, and this rod may be looked upon as the "hinge" around which the basilar membrane moves (Wilkinson & Gray, 1924).

Gray's measurements of the cochlea are the following (see Fig. 1): diameter of lowest whorl of cochlea (*A–B*); diameter of second whorl of cochlea (*C–D*);

slant height of cochlea (*E–F*). In addition, the number of turns of the cochlea in each species is given. The "slant height of cochlea" is defined as the distance from the upper margin of the oval window to the apex of the organ. There is no difficulty in making corresponding measurements on the sectioned cochleae, and it appeared to be of some value to compare the two sets of figures. Gray's figures are given in Table II, my own corresponding ones in Table III.

Table II. *Measurements from Gray's* Labyrinth of Animals (*in millimetres*)

	Diameter of lowest whorl of cochlea	Diameter of second whorl of cochlea	Slant height of cochlea	Number of turns of cochlea
Human	8·25	4·62	7·0	2¾
Cat	6·0	3·5	4·25	3+
Sheep	7·0	3·5	5·0	2¼
Calf		Not given		
Dog	6·25	3·5	4·75	3¼
Guinea-pig		Not given		
Mouse	1·5	0·75	1·5	2

Table III. *Corresponding measurements obtained from sections of cochleae* (*in millimetres*)

	Diameter of lowest whorl of cochlea	Diameter of second whorl of cochlea	Slant height of cochlea	Number of turns of cochlea
Human	6·0	4·0	6·0	2½
Cat	4·5	2·5	4·25	3
Sheep	7·5	4·0	6·0	2
Calf	5·5	4·0	6·0	2
Dog	5·0	3·5	5·0	3
Guinea-pig	3·5	2·3	5·0	4½
Mouse	1·4	—	1·65	1½

DISCUSSION

It is impossible not to feel surprise at the extraordinary resemblance in the dimensions of the scala media in man and in the six other mammalian species which were examined (Fig. 2). The resemblances in the width of the basilar membranes are equally striking (Table I). The size of this inner ear duct appears to have very little relation to body size as a whole. Organs such as the eye or related parts of the central nervous system show differences which bear a much closer relation to absolute body size.

The part of the inner ear which contains the sense organ of hearing is nearly as large in cross section in the guinea-pig as in man or calf. The mouse appears to be an exception. Even so, the cross section of the scala media in the mouse is approximately two-thirds of that of the guinea-pig, and the width of the basilar membrane in the basal coil of the mouse is 0·13 mm. as against 0·20 mm. in the corresponding position in the human cochlea.

In the resonance theory of hearing attention is generally directed to two factors: (*a*) the width of the basilar membrane, i.e. the length of the fibres making up the basilar membrane; (*b*) the state of tension of these fibres, which is said to be dependent on the width of the spiral ligament. This ligament is the structure seen at the outer edge of the basilar membrane, stretching between the basilar membrane and the outer endosteum of the bony canal. It is wide at the base and thins away towards the apex (Fig. 1). By taking into account the differentiation of the basilar membrane, both as to the length of the fibres, and their state of tension, it is possible to explain on physical grounds the tuning of the fibres for the whole range of tones comprised in the audible scale, some 10–11 octaves (Wilkinson & Gray, 1924).

We have very little knowledge about the range of hearing in animals. Pavlov's experiments with conditioned reflexes suggest that dogs have an extensive range of hearing, perhaps more extensive than ours (Pavlov, 1927; Beatty, 1932). On the present findings one is tempted to speculate that the mouse has a high-pitched range of hearing; it may well be that its cochleae in the upper registers are sensitive to pitches so high as to be inaudible to the human ear. But such a speculation involves the acceptance of some form of resonance theory. The high-pitched squeaks of mice, almost inaudible to our own ears, would lend support to such a view.

No animal can escape from sound. An elaborately coiled cochlea became evolved in mammals, and evidently the fundamental plan tends to be the same whatever the size of the animal. Once the organ was evolved, nature allowed the minimum of variation. The remarkable correlation in actual size in different species suggests that the cochlea is set apart not only for the perception, but also for the analysis of sound vibrations; a strong point in favour of the resonance theory. It is hoped to extend this study in order to present a more complete survey of the inner ear in the mammalian series.

I wish to thank Prof. A. B. Appleton for his many helpful suggestions and advice in the preparations of this paper, and for the facilities he has afforded me in the Anatomy Department of St Thomas's Hospital Medical School. I also have to thank Mr F. J. Cleminson, the Director of the Ferens Institute of Oto-Laryngology for his kind permission to examine the many beautiful preparations of human and animal cochleae at that Institute, and Mr W. Pilgrim, the senior technician, for his help with the microphotographs.

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FURTHER OBSERVATIONS ON THE EFFECTS OF SEX HORMONES ON THE PROSTATE AND SEMINAL VESICLES OF MONKEYS

By S. ZUCKERMAN and O. C. SANDYS
From the Department of Human Anatomy, Oxford

Oestrogenic stimulation increases the size of the accessory reproductive organs of male macaques (Parkes & Zuckerman, 1935; Courrier & Gros, 1935; van Wagenen, 1935). In the prostate the most extensive growth occurs in the part that intervenes, above the level of the openings of the ejaculatory ducts, between the glandular zone behind and the urethra in front (Zuckerman, 1938). As the fibro-muscular and epithelial tissues of this region (called for convenience either utricular bed or middle lobe) proliferate, the prostatic glands are displaced dorsolaterally and become relatively reduced in number until they form a capsule to the hypertrophied middle lobe, the fibromuscular tissue of which, it may be noted, is presumably derived, either wholly or partly, from the mesoderm of the caudal end of the genital cord. The effect of oestrogenic stimulation on the seminal vesicles is equally striking. The glandular epithelium undergoes atrophy until, if the stimulation continues long enough, the vesicular lobules become converted into thick-walled muscular tubes lined by a simple non-folded epithelium.

Androgenic (male hormone) stimulation has an opposite effect on both the prostate and seminal vesicles. In the former it acts mainly on the epithelium of the glandular zone (Zuckerman & Parkes, 1938), producing changes which reproduce the normal growth of the organ (Zuckerman, 1938). In the seminal vesicle it acts most intensively on the glandular epithelium, for whose complex folding it is responsible. These reactions, and those of the accessory organs to stimulation by various combinations of the sex hormones, are quantitatively analysed in the following paper, which thus provides data on the differential development of the glandular and fibromuscular tissue of the male reproductive tract in monkeys. These new facts bear on the problem of the aetiology and treatment of benign enlargement of the prostate.

The paper also provides further information about the responses of the prostate of the Hanuman langur (*Presbytis entellus*) to prolonged oestrogenic stimulation. In an earlier communication (Zuckerman & Parkes, 1936*a*) it was pointed out that the uterus masculinus in this species differs from that of most other monkeys in being a glandular organ, and by responding to oestrogenic stimulation, not by squamous metaplasia, but by true glandular hyperplasia. The experiment on which this observation was based lasted only

39 days, and it was therefore suggested that were the hyperplasia to continue for a longer period, the prostate might be so transformed as to present in the utricular bed an appearance similar to that of a fibroadenoma in the middle prostatic lobe in man. Though this prediction has not been fulfilled, the results obtained in long-term experiments on this species are of great interest.

THE RHESUS MONKEY, *MACACA MULATTA*

Material and methods

Sections of the prostate and seminal vesicles of normal rhesus monkeys and of rhesus monkeys that had been injected with (*a*) oestrone, (*b*) various preparations of male hormone, and (*c*) oestrone and progesterone were available for study. According to Schultz's tables for body weight and dentition in this species of monkey (1933, 1935), and with the exception of a mature normal and a mature castrated male rhesus, none of the animals was older than 36 months. According to Schultz (1933) rhesus monkeys have not by then reached puberty, and histological examination of the testes of the animals concerned showed that they were all sexually immature. In this way animals were excluded whose accessory reproductive organs had been subjected to "natural" male-hormone stimulation.

In order to limit the amount of tabular material presented in this paper, details of the experimental treatment received by each individual animal are omitted. Comparisons have been made mainly between animals which were injected for 13, 14 or 15 days. The treatment given to each *group* of animals (Table I) was as follows:

Prostate

Six animals injected with oestrone. The estimated ages of the animals varied between 24 and 36 months. Two of the animals received 50γ and four 100γ of oestrone daily for either 14 or 15 days.

Six animals treated with testosterone propionate. The estimated ages of the animals varied between 16 and 28 months. The monkeys received the hormone over a period of 14 days, the total amount administered varying between 200 and 400 mg.

Seven animals injected with both oestrone and male hormone. The estimated ages of the animals varied between 24 and 36 months. All the animals were injected for 14 days, and each received 100γ of oestrone daily. Three were given a total of 35 mg. of testosterone during this period. Three were injected with testosterone propionate (10, 25 and 50 mg. respectively), and one was given 70 mg. of androstanediol.

Seven animals injected with both oestrone and progesterone. The estimated ages of the animals varied between 20 and 35 months. All the animals were injected for 14 days, and each received either 50 or 200γ of oestrone daily. During this experimental period three were given 2·8, one 5·6, two 7·0 and the seventh 10·5 mg. of progesterone.

Seminal vesicle

Nine immature normal monkeys. The estimated ages of the animals from which control tissues were taken varied between 18 and 34 months.

Eighteen animals injected with oestrone. The estimated ages of the animals varied between 15 and 36 months. The period of injection varied between 6 and 368 days; eight animals were injected for 14 days. The amount of hormone given each animal daily varied between 50γ and 5 mg., the usual amount being 100γ.

Twelve animals injected with male hormone. The estimated ages of these animals varied between 16 and 28 months. The period of injection varied from 10 to 28 days. Six animals received testosterone propionate, in amounts varying between 200 and 400 mg., over a period of 14 days. Three of the other animals were given androsterone (55, 100 and 110 mg.) and three androstanediol (60, 100 and 140 mg.).

Nine animals injected with oestrone and male hormone. The estimated ages of these animals varied between 24 and 36 months. Each received 100γ of oestrone daily for 14 days. During this period three were given testosterone propionate (10, 25 and 50 mg.); three were given testosterone (35 mg. each); and three, androstanediol (70 mg. each).

Six animals injected with oestrone and progesterone. The estimated ages of these animals varied between 20 and 35 months. Three were given 50γ and three 200γ of oestrone daily for 14 days. Progesterone was also injected daily, three receiving a total of 2·8 mg., one 5·6 mg., one 7·0 mg. and the sixth 10·5 mg. during the experimental period.

In all the experiments referred to above, injections were made intramuscularly in oil solution, and, except in the case of testosterone propionate, were given once daily. The testosterone propionate was given at 2, 3 or 6 day intervals.

Measurement of accessory organs

The areas of the glandular, utricular and urethral parts of transverse sections of the prostate, taken at the level of the middle of the vagina masculina, were measured in the manner already described (Zuckerman 1938) from projections on to squared paper.

In order to avoid unconscious selection, sections of the seminal vesicles were projected on to paper on which a triangle of arbitrary shape had been drawn. Those "tubules" which fell on, or were nearest to the apices of the triangle were traced in outline, and the line of junction of glandular and muscular tissue was drawn. By means of a planimeter the cross-sectional area of each "tubule" was measured. The difference between the total cross-sectional area of each "tubule", and of the area lying within the line of junction of muscular and epithelial tissue, gave a measure of the amount of muscle in each "tubule". The means of the total areas, and of the areas of gland and muscle in the three "tubules", were calculated and, from these values, the average percentage area of muscle to the whole "tubule" was obtained.

Results

The prostate

The normal prostate.

The mean dimensions of mid-utricular transverse sections of prostates of normal and of experimental monkeys of estimated age not greater than 36 months are collected in Table I.

Control data regarding the growth changes in the prostate of normal

rhesus monkeys are summarized diagrammatically in Figs. 1 and 2, and are discussed elsewhere in detail (Zuckerman, 1938). They have been incorporated in these figures for purposes of comparison.

Table I. *Mean dimensions of mid-utricular transverse sections of the prostate of normal and experimental rhesus monkeys of estimated age not greater than* 36 *months*

Class	No. of specimens	Whole organ (actual, sq. mm.)	% glandular area	% utricular bed	% urethra
Normal	9	25·8±1·40	55·9±2·01	9·1±1·17	35·0±2·13
Oestrone (14 days)	6	65·8±6·89	59·6±5·11	14·0±4·21	26·4±2·24
Testosterone propionate (14 days)	6	84·3±7·99	77·7±2·46	4·0±0·90	18·3±1·76
Oestrone and male hormone (14 days)	7	48·4±1·97	71·3±1·44	5·8±0·91	22·9±1·63
Oestrone and progesterone (14 days)	7	46·9±3·66	57·6±3·13	19·7±2·13	22·7±1·62

Table II. *Dimensions of mid-utricular transverse sections of the prostate in a mature castrated and in a mature normal rhesus monkey*

Animal	Condition	Whole organ (actual, sq. mm.)	% glandular area	% utricular bed	% urethra
149	Normal	216·9	84·3	2·2	13·5
209	Castrated	90·3	81·0	2·7	16·3

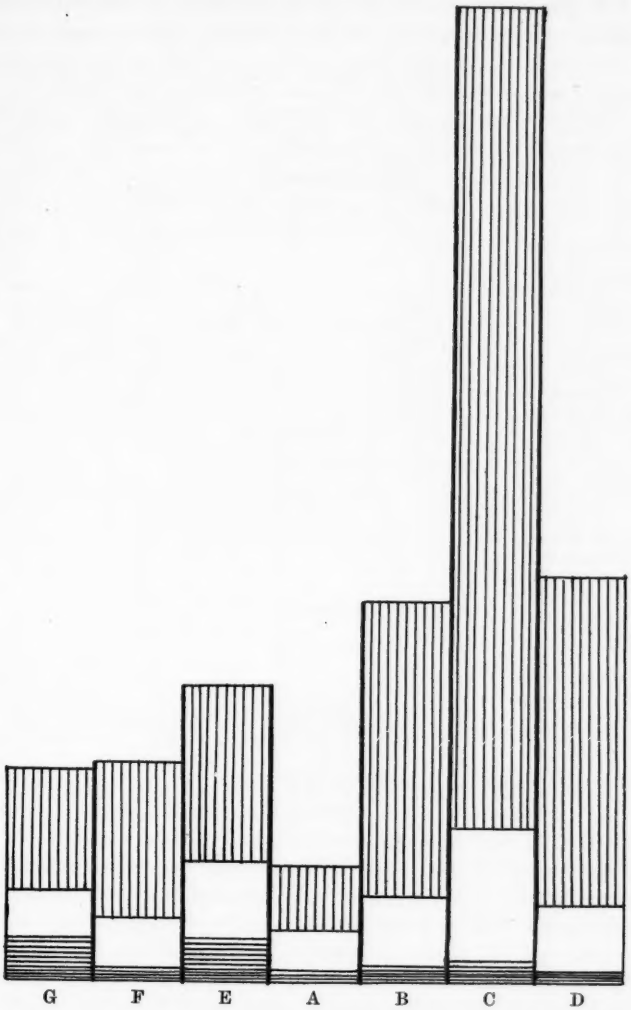
The effect of castration.

The prostate of a mature rhesus monkey (209) that had been castrated 5 months before autopsy was studied. The individual glandular acini are mostly involuted, and their secretory cells are much lower than normal. The whole gland is also considerably shrunken, but the ratio of glandular tissue to the utricular bed and to the urethral part of the organ, and that of the latter two to each other, are approximately the same as in the normal mature prostate (Table II, and Text-figs. 1, 2). Whether or not these proportions would alter in a longer period of castration, and revert to the conditions which prevail in younger rhesus monkeys (Table I), is still to be determined.

The injection of male hormone into a mature castrated rhesus restored the prostate to its normal size and character (Zuckerman & Parkes, 1938).

The effect of oestrone on the proportions of the prostate.

The present series of observations is new, and relates only to monkeys not older than 36 months which were injected with oestrone for 14 or 15 days (Table I). The observations were made in order to obtain data that would be comparable with those obtained from animals which were injected for an



Text-fig. 1. The mean actual proportions, drawn to the same scale, of the prostate in different groups of rhesus monkeys. Horizontally lined area = utricular bed; clear area = urethra; vertically lined area = glandular zone. A, nine normal monkeys whose estimated age was not greater than 36 months. B, six monkeys of similar age after 14 days' treatment with testosterone propionate. C, a normal mature rhesus monkey. D, a castrated mature rhesus monkey. E, six monkeys whose estimated age was not greater than 36 months after 14 or 15 days' treatment with oestrone. F, seven monkeys of the same age after 14 days' treatment with both oestrone and testosterone. G, seven monkeys of the same age after 14 days' treatment with both oestrone and progesterone.

Table III. *The significance of the differences between the mean dimensions of the prostate in various groups of immature rhesus monkeys*

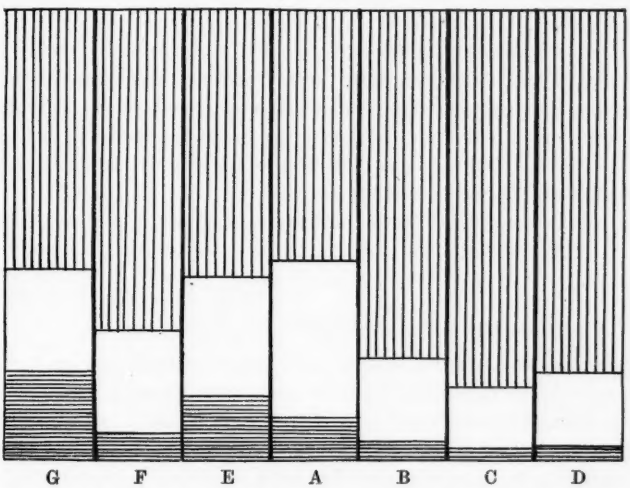
Comparison of the mean prostatic dimensions in monkeys given	With the mean prostatic dimensions in monkeys given			
	Oestrone (*P*)	Testosterone propionate (*P*)	Oestrone and male hormone (*P*)	Oestrone and progesterone (*P*)
	(1) Whole organ			
No treatment	<0·01	<0·01	<0·01	<0·01
Oestrone	—	0·1	0·02	0·02
Testosterone propionate	—	—	<0·01	<0·01
Oestrone and male hormone	—	—	—	0·7
	(2) Percentage glandular tissue			
No treatment	0·5	<0·01	<0·01	0·6
Oestrone	—	<0·01	0·05	0·7
Testosterone propionate	—	—	0·05	<0·01
Oestrone and male hormone	—	—	—	<0·01
	(3) Percentage utricular bed			
No treatment	0·1	<0·01	0·05	<0·01
Oestrone	—	0·02	0·05	0·2
Testosterone propionate	—	—	0·2	<0·01
Oestrone and male hormone	—	—	—	<0·01
	(4) Percentage urethra			
No treatment	0·02	<0·01	<0·01	<0·01
Oestrone	—	<0·01	0·2	0·2
Testosterone propionate	—	—	0·1	0·1
Oestrone and male hormone	—	—	—	0·9

equal time with other sex hormones. The nature of the changes caused by oestrone has been discussed in detail elsewhere (Zuckerman, 1938). The present results are shown diagrammatically in Text-figs. 1 and 2.

The significance of the differences between the mean actual size of the whole prostate and the mean relative sizes of the glandular area, utricular bed, and urethra in the various groups of animals listed in Table I has been analysed by means of Fisher's "*t*" test (1932). The results of the analysis are collected in Table III. Only values of *P* equal to or less than 0·01 are regarded as indicating significant differences. It is clear from Table III that a significant increase occurs in the size of the whole prostate in 14 days of oestrogenic stimulation, but that significant alterations in the relative extent of the different parts of the prostate do not occur within that time. The mean value for the relative extent of the glandular zone is actually larger in this group than in the controls, but as is clear from data already published (Zuckerman, 1938) the position becomes significantly reversed if the injections are continued for longer than 14 days.

The effect of testosterone propionate on the proportions of the prostate.

Male hormone causes growth in all parts of the prostate, but the relative extent of both the urethral area and utricular bed becomes significantly less, as that of the glandular zone increases (Tables I, III, Text-figs. 1, 2). A similar change occurs during normal growth, when the prostate is influenced by the male hormone produced by the testes (cf. Text-figs. 1, 2). These experimental changes become significant statistically within 14 days of testosterone propionate treatment, and comparison shows that while under the conditions of the present experiments male hormone does not stimulate significantly more



Text-fig. 2. The mean relative proportions of the prostate in the same groups of rhesus monkeys detailed in Text-fig. 1. Different areas as in Text-fig. 1.

prostatic growth within this period than does oestrone (Table III), it does cause changes in the relative extent of both the glandular and urethral areas which are significantly different (and in the utricular bed almost significantly different) from those produced by oestrone.

The effect of oestrone and male hormone given simultaneously.

It has been clearly established that under certain conditions the effects of male hormone may be neutralized by the simultaneous administration of oestrogenic hormone (de Jongh, 1935; Zuckerman & Parkes, 1936*b*; Korenchevsky & Dennison, 1935; Courrier & Cohen-Solal, 1937; Rusch, 1937). Under other conditions there may be synergic activity of the two types of hormone (Freud, 1933; Korenchevsky & Dennison, 1934, 1935; Overholser & Nelson, 1935). Tables I and III show that, while the prostate of animals which have

been treated with both hormones is about twice normal size, it is significantly smaller than that of monkeys injected for 14 days with male hormone alone, and almost significantly smaller than the prostate of monkeys injected with similar amounts of oestrone alone. The tables also show that the relative extent of the glandular area in the animals treated in this way is significantly greater than that of normal animals, while that of the urethral area is significantly less. This fact shows that under the conditions of the present experiments the effects of the male hormone were dominant. This conclusion is also suggested by the fact that there is no significant difference in the relative extent of the various parts of the prostate in the animals treated with male hormone alone and in those treated with male hormone and oestrogenic hormone together. Conversely the utricular bed in the animals which received both hormones is almost significantly smaller than in those which received oestrone alone, while the glandular area is almost significantly greater—a finding which indicates the suppression of oestrogenic effects. The fact that the actual metaplastic changes normally produced by oestrone in the vagina masculina of rhesus monkeys are inhibited by male hormone has already been commented upon (Zuckerman, 1936*a*).

These new observations thus indicate a mutual inhibition, and not a synergism, of the partial effects of oestrogenic and androgenic stimulation on the prostate.

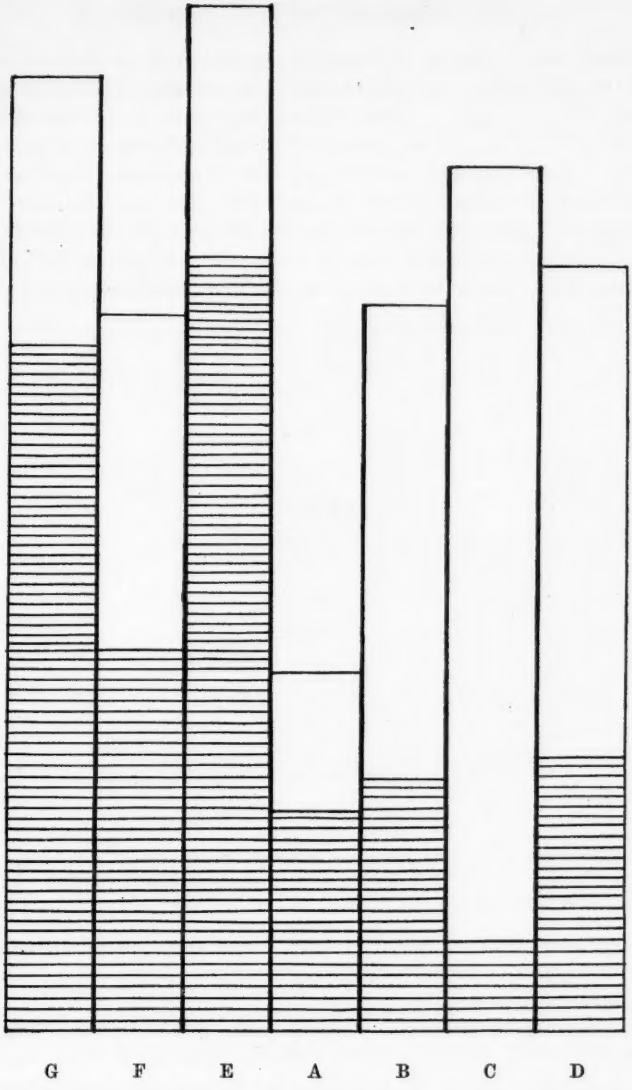
The effect of oestrone and progesterone given simultaneously.

The prostate of monkeys treated with both oestrone and progesterone is significantly larger than normal. It is almost significantly smaller than the prostates of monkeys injected with oestrone alone, and is not significantly different from the prostates of monkeys treated with both oestrogenic and male hormones. It appears from Tables I and III, however, that in the amounts given, progesterone is far less able to inhibit the effects of oestrogenic stimulation than is male hormone. Thus the changes which occur in the proportions of the three parts of the prostate are not significantly different from those produced by oestrogenic stimulation alone. In this respect progesterone in the proportions given (maximum 20 : 1) clearly has a weaker inhibitory effect on oestrogenic stimulation of the prostate than has male hormone. Since progesterone in higher ratios is fully able to inhibit oestrogenic effects in female mammals, it is likely that in higher doses it would do the same in males as well.

The seminal vesicles

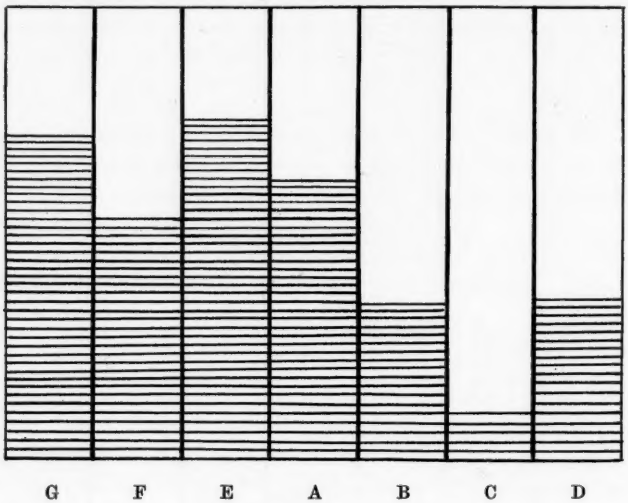
Normal growth.

During normal pubertal development, the lobules of the seminal vesicles grow rapidly. Their cross-sectional diameter increases, and their muscular walls become relatively thinner. Coincidentally, the glandular tissue becomes relatively more extensive, the epithelium more complexly arranged, and the individual cells larger.



Text-fig. 3. The mean cross-sectional area of a tubule of the seminal vesicle in each of seven different groups of rhesus monkeys and all drawn to the same scale. Horizontally lined area = muscular wall; clear area = epithelial tissue and lumen. A, nine normal monkeys whose estimated age was not greater than 36 months. B, six monkeys of similar age after 14 days' treatment with testosterone propionate. C, a normal mature rhesus monkey. D, a castrated mature rhesus monkey. E, eight monkeys whose estimated age was not greater than 36 months after 14 days' treatment with oestrone. F, nine monkeys of the same age after 14 days' treatment with both oestrone and testosterone. G, six monkeys of the same age after 14 days' treatment with both oestrone and progesterone.

The main growth change is displayed quantitatively in the first two lines of Table IV, and in Text-figs. 3 and 4. The table and figures also give the mean total areas and the relative extent of muscular tissue in the tubules of the seminal vesicles of the various groups of animals referred to on p. 599. The method used in determining the relative extent of muscular tissue has already been described. It should be noted again that the "muscle-index" ("percentage muscle") gives the relative amount of the total cross-sectional area of the vesicular tubules that is made up of muscle, and not the ratio of muscle to glandular tissue. The latter would have provided a better index of the changes



Text-fig. 4. The mean relative proportions of a tubule of the seminal vesicle in the same groups of monkeys detailed in Text-fig. 3. Horizontally lined area = ratio of muscular coat to cross-sectional area of tubule.

which take place in the seminal vesicles, but it is practically impossible to estimate owing to the difficulty of determining the cross-sectional area of the complex lumen of the tubules.

Effect of castration.

The qualitative effects of castration on the seminal vesicles have been described elsewhere (Zuckerman & Parkes, 1938). A quantitative measure of the changes is given in Table IV, and in Figs. 3 and 4, from which it appears that in a period of 5 months after castration, the relative increase of the muscular wall is disproportionately greater than the decrease in the actual size of the tubules. Examination of the seminal vesicles of a castrated monkey after they had been restored to their normal condition by means of male hormone shows that there was a fourfold increase in the size of the tubules

Table IV. *Mean dimensions of cross-sections of seminal vesicle tubules in normal and experimental rhesus monkeys of estimated age not greater than 36 months. In all groups the period of injection varied between 13 and 15 days*

Class	No. of specimens	Mean total area, sq. mm.	% muscle
Immature normal	9	0·80 ±0·11	61·6 ±3·45
Mature normal	1	1·93	10·4
Mature castrate (5 months)	1	1·71	35·7
Male hormone (injected 14 days)	6	1·62 ±0·15	34·5 ±2·00
Oestrone (injected 14 days)	8	2·29 ±0·34	75·5 ±3·34
Oestrone and male hormone (injected 14 days)	9	1·60 ±0·12	53·3 ±3·79
Oestrone and progesterone (injected 14 days)	6	2·13 ±0·39	71·7 ±3·32

Table V. *The significance of the differences between mean cross-sectional dimensions of vesicular lobules in various groups of immature rhesus monkeys*

Comparison of the mean dimensions of vesicular lobules in monkeys given	With the mean dimensions of vesicular lobules in monkeys given			
	Male hormone (P)	Oestrone (P)	Oestrone and male hormone (P)	Oestrone and progesterone (P)
		Total area		
No treatment	<0·01	<0·01	<0·01	<0·01
Male hormone	—	0·1	0·9	0·2
Oestrone	—	—	0·05	0·8
Oestrone and male hormone	—	—	—	0·2
		Percentage muscle		
No treatment	<0·01	0·02–0·01	0·1	0·05
Male hormone	—	<0·01	<0·01	<0·01
Oestrone	—	—	<0·01	0·4
Oestrone and male hormone	—	—	—	<0·01

as a result of the injections, and that the muscle index had been restored to normal.

Effect of oestrone on the seminal vesicles.

The changes which occur in the seminal vesicles as a result of oestrogenic stimulation have been qualitatively described in the introduction to this paper. Table IV shows that the diameter of the lobules undergoes a mean increase of almost three times as a result of 14 days' oestrogenic stimulation (50 or 100γ daily). The muscle-index also increases, but not significantly, in this period of time (Table V and Text-fig. 4). If the stimulation is prolonged sufficiently, the change does become significant, a fact which was revealed in experiments in which daily oestrogenic injections were continued for 62 days (100γ daily), 84 days (200γ daily), 365 days (10γ daily), and 368 days (100γ daily).

Effect of male hormone on the seminal vesicles.

The mean cross-sectional diameter of the vesicular lobules of monkeys injected for 14 days with testosterone propionate is significantly greater than normal (Tables IV and V). On the other hand, it is much smaller than in monkeys which have been an equal length of time under the influence of oestrogenic stimulation (when the total amounts of oestrone administered varied from 1/150 to 1/640th part of the total amount of testosterone propionate injected). On account of the size variations within the two groups, this latter difference is not significant statistically. That the increase in the size of the lobules when they are under the influence of androgenic stimulation represents a completely different process from the growth which occurs under the influence of oestrogenic stimulation is indicated by the fact that the actual amount of muscle in the seminal vesicles of immature monkeys after 14 days' stimulation by male hormone is little greater than in normal animals, whereas it is increased almost fourfold after 14 days of oestrogenic stimulation (Text-fig. 3). The increase in the diameter of the tubules as a result of androgenic stimulation may therefore be almost entirely attributed to growth in the glandular epithelium and in the size of the lumen.

Effect of oestrone and male hormone on the seminal vesicles.

The data given in Table IV and analysed in Table V show that while much smaller, the mean cross-sectional area of the seminal vesicle tubules of young monkeys injected for 14 days with both male hormone and oestrone is not significantly different from the cross-sectional area in monkeys injected with oestrone alone. The area is almost the same as in monkeys treated for an equal length of time with male hormone alone (Text-fig. 3). On the other hand, the muscle index in the animals which received both androgenic and oestrogenic stimulation is intermediate between the indices for the two groups of animals which received the hormones separately (Text-fig. 4), and significantly different from both (Table V). The data thus suggest that in the proportions given, oestrone and male hormone mutually and partially inhibit each other's effects on the seminal vesicles.

The effects of oestrone and progesterone on the seminal vesicles.

The data given in Tables IV and V show that, in the proportions given, progesterone does not inhibit the effects of oestrogenic stimulation upon the seminal vesicles.

THE HANUMAN LANGUR, *PRESBYTIS ENTELLUS*

Material

The reproductive tracts of two normal immature Hanuman langurs were available as controls. In both animals the first molar was the only permanent tooth that had erupted. One animal (263) weighed 2940 g., and the other (201) 3740 g.

Two other immature Hanuman langurs of similar age were injected daily and intramuscularly with an oil solution of oestrone for 483 and 316 days respectively. No. 202, which was injected for the longer period, was castrated on the 31st day of the experiment. Its maximum body weight during the period of observation was 6090 g., and at the end of the experiment its permanent incisors and second permanent molars had erupted. With the exception of the first 13 days, during which 400γ of oestrone were administered daily, the animal received 600γ each day, the total amount given during the 483 days of the experiment being 287·2 mg. Apart from enlargement and coloration of the nipples, no external changes occurred as a result of the injections, and there was no retention of urine.

The permanent central incisors of the second experimental animal (205) were erupting at the end of the experiment, and its maximum body weight was 4900 g. Over the greater part of the experiment it was given 400γ of oestrone daily, the total amount injected being 121·5 mg. Apart from changes in the nipples, no external effects of the treatment were observed.

For comparison with these prostates there were also available those of two other Hanuman langurs which had been injected bi-weekly with testosterone propionate for 142 and 157 days respectively. Both animals were the same dental age as the controls, and the maximum body weight of the first, which received a total of 0·985 g. of the hormone, was 4390 g., while that of the second, which received 1·985 g., was 4590 g.

Results

The cranio-caudal, transverse and dorso-ventral diameters of the oestrogen-stimulated prostates are some 50 % larger than the controls, but slightly smaller than those of the two prostates which had grown under the influence of androgenic stimulation. They are also slightly larger than those of the prostate of the entellus monkey that has already been described (Zuckerman & Parkes, 1936*a*), and which was injected with oestrone for only 39 days. While the small number of specimens available for this study precludes detailed statement, analysis by the methods used with the rhesus monkey shows that oestrogenic and androgenic stimulation induce the same general prostatic changes in the langur that they do in the rhesus macaque. Thus oestrogenic stimulation decreases the relative extent of the glandular zone and markedly increases that of the utricular bed, an effect which is the opposite of that of androgenic stimulation, while, as in the rhesus, the relative extent of the urethral zone remains approximately the same after oestrogenic stimulation, whereas it diminishes as a result of treatment with male hormone

The true prostatic glands in the animals which were injected with oestrone retain their infantile character and in no part of the organ do they show metaplastic changes. Those in the two animals injected with male hormone show changes that are identical with those which occur in the rhesus monkey after treatment (Zuckerman & Parkes, 1938). The only effects of oestrogenic

stimulation that require special mention are those which occur in the uterus masculinus, in the terminal parts of the ejaculatory ducts, in the prostatic collecting ducts, and in the urethral epithelium.

Uterus masculinus

The uterus masculinus is smaller in the controls than it is either in the two monkeys which were given androgenic hormone or in the two which were injected with oestrone. Its growth under the influence of male hormone must be either regarded as a consequence of general growth in the prostate or as a specific effect of androgenic stimulation upon tissue derived from the Müllerian ducts, similar, for example, to that which has been reported by some workers in the case of the uterus itself (Klein & Parkes, 1937; Robson, 1937; Courrier & Gros, 1938).

In the normal uninjected immature langur the uterus masculinus has the same general form as that of the oestrone-injected langur (MM 56) already described (Zuckerman & Parkes, 1936*a*). In the main it consists of a sagitally disposed bar of deeply-staining epithelial cells which in places are differentiated to form small secreting acini lined with columnar cells. There are, however, fewer acini in the uninjected controls, and also fewer peninsular extensions of the main cord of cells, which is also imperfectly canalized (at its urethral end the bar appears to be hardly canalized at all). In one of the two control specimens the uterus masculinus ends above in a cystic expansion. In the other it ends as a small tubular gland, isolated traces of which can be recognized at two more cranial levels of the prostate.

The uterus masculinus of the langur (MM 56) which was injected with oestrone for 39 days is about 50 % longer than normal and showed signs of active proliferation (as indicated by numerous mitoses, by many irregular peninsular extensions of the deeper staining utricular cells, by numerous secreting acini, and by the enclosure of utricular stroma within the peninsular extensions). That of the langur (205) which was injected for 316 days is very similar in appearance, except in the region of its mouth (see below) and at its cranial end, which is an isolated cyst whose maximum diameter is 0·83 mm. In no part of its extent has it undergone squamous metaplasia of the kind which occurs in male rhesus monkeys as a result of oestrogenic stimulation. The uterus masculinus of the langur (206) which was injected for 483 days is very different, although it too shows no sign of squamous metaplasia. It consists of a sac-like diverticulum, somewhat more than twice the length of the normal uterus masculinus, and it is lined by flattened columnar epithelium. This layer of cells covers an irregular stratum of typical basal utricular cells, within which several small acini lined by secreting columnar cells have differentiated.

A similar sac-like uterus masculinus is present in the langur (206) which was injected with testosterone propionate over a period of 157 days. It is,

however, about 20 % shorter, and possesses fewer secondary acini. The uterus masculinus in the second langur which was given male hormone is similar to, but smaller than, that of MM 56 and 205, and it is noteworthy that the acini in this specimen were actively secreting.

The utricular stroma, like all the non-epithelial tissue of the prostate, appears to be much more oedematous in the animals which were given oestrone than in either the normal langurs or in the two which were given male hormone.

Common ejaculatory ducts

In the normal immature langur the common ejaculatory ducts open into the urethra on either side of and at about the same level as does the uterus masculinus. The lining epithelium of the ducts in one (201) of the two normal specimens studied is in some places cuboidal, in others columnar, and in general is at most two layers of cells deep. In the other animal (263) it is deeper, and glandular acini of the kind normally present in the uterus masculinus are occasionally differentiated in the region between the opening of the duct and the upper blind end of the uterus masculinus, while in other places the lining columnar cells are much enlarged and so multiplied as almost to occlude the duct (cf. Pl. I, fig. 2).

Glandular differentiation of the same kind is not present in either of the two langurs which were injected with testosterone propionate, and in them the epithelium is a normal simple columnar type. It occurs, however, in the langur (MM 56) which was injected with oestrone for 39 days (Zuckerman & Parkes, 1936*a*), while "mucinous hyperplasia", of the kind seen in the one normal specimen, has taken place in the epithelium of the terminal parts of the ejaculatory ducts of both langurs which were injected with oestrone for longer periods. This change is best marked in 202, in which the transformation of the typical lining columnar into large mucoid cells (Pl. I, fig. 2) has in places proceeded so far as almost to block the lumen. Squamous metaplasia of the kind which occurs in the terminal parts of the ejaculatory ducts of rhesus monkeys as a result of oestrogenic stimulation (Zuckerman, 1938) did not occur in any of the langurs.

Collecting ducts of the prostate

In the langur the collecting ducts of the prostate open on the crista urethralis and into the prostatic sinuses both below and above the opening of the uterus masculinus. The openings are confined to a region which comprises about a third of the cranio-caudal diameter of the prostate, and they reach far closer to the lower pole of the organ than they do its upper surface. The upper collecting ducts thus descend (to a level well below the blind end of the uterus masculinus), and the lowermost ones ascend to open into the urethra. In series with the lowermost ducts are a number of tubular urethral glands which have no connexion with the prostatic glands proper. They open on all sides into the urethra, and their lining epithelium is similar to that of the collecting

ducts. A few ventral urethral glands, which also have no connexion with the prostatic glands proper, are occasionally present in upper levels of the prostate.

Most of the collecting ducts in the normal control specimens are distended and contain secretion, and throughout their course their lining epithelium is generally a single layer of somewhat flattened and irregular columnar epithelium. Like the more caudal urethral glands, however, they may occasionally be lined by two layers of cells, of which the inner is much the more prominent. The stroma immediately surrounding the ducts is oedematous.

Although the columnar cells of the lining epithelium are both larger and obviously more active, the collecting ducts of the prostates of the two langurs which were injected with testosterone propionate appear less prominent than in the control specimens owing to the encroachment of the glandular zone on the urethral area, and the consequent relative shrinkage of the utricular bed. With the growth of the latter region, and the dorsolateral displacement of the glandular zone, the ducts, in contrast, appear much more prominent in the prostates of the langurs which were injected with oestrone. They are also more distended with secretion, and their epithelium is in general flatter than normal.

In the latter two specimens the epithelium of some of the ducts has undergone hyperplasia in the region of the mouth of the uterus masculinus. In one (205) the change shows itself merely by an occasionally increased stratification of the duct epithelium, which in places is as many as five cells deep. A similar increase in the stratification of the ducts has been reported to occur in man as a result of oestrogenic stimulation (Moore & McLellan, 1938). In the other langur (202) a few glandular acini have differentiated within the thickened duct walls (Pl. I, figs. 3–5), from which in some cases they have budded as tubular glands. This change is confined to the terminal parts of the ducts.

Urethral epithelium

The change in the terminal parts of the collecting ducts would appear to be related to a process of hyperplasia which takes place on the summit of the crista urethralis as a result of oestrogenic stimulation. Thus, instead of being some four to six cells deep, the urethral epithelium in this region is in some places deeply stratified in both 202 and 205, and peninsular blocks of cells extent into the stroma. The change is not uniform and isolated parts of the crista are covered by normal epithelium (Pl. I, fig. 1). The caudal end of the uterus masculinus is involved in the process of stratification, and because of it, it is impossible to demarcate the limits of what should be regarded as the utricular mouth.

The hyperplasia of the epithelium of the crista urethralis, which according to Moore & McLellan (1938) also occurs in man as a result of oestrogenic stimulation, is not associated with superficial keratinization and desquamation, as it is in the rhesus macaque. Nor does this latter change (which takes

place throughout the urethral epithelium of the rhesus, with the exception of the region cranial to the openings of the uterus masculinus and ejaculatory ducts) occur anywhere in the urethra of the langur. After more than a year's stimulation by oestrogenic hormone, the latter thus shows practically no abnormal changes, while a further change which is only slightly manifested by the langur, and which is very pronounced in the rhesus monkey after oestrogenic stimulation (Zuckerman, 1938), is the occurrence of lymphocytic nodes deep to the urethral epithelium and in the utricular zone of the prostate.

It may be noted that the urethral epithelium in the langur, which normally has many more glandular offshoots than that of the rhesus macaque, shows no abnormal changes after androgenic stimulation, and that in some regions it appears to become thinner than normal.

DISCUSSION

The impact of recent experimental findings upon the clinical problem of benign enlargement of the prostate has shown itself mainly in a renewal of interest in pathological investigation of operative and post-mortem material, and in attempts to control the disease by means of endocrine treatment. In a series of recent papers Cunéo (1936, 1938) and Cunéo & Jomain (1938) advocate the view that the fibroadenomatous changes in the middle (prespermatic) lobe, which lead to the enlargement, begin as an irregular hyperplasia of the uterus masculinus that results from a "senile" decrease in the production of male hormone. The view that the uterus masculinus is implicated in the process, which was first formulated and elaborated on the basis of experimental work on monkeys (Parkes & Zuckerman, 1935; Zuckerman, 1936*b*, 1938), still lacks a thorough experimental basis, for, contrary to expectation (Zuckerman & Parkes, 1936*a*), the observations reported in the present paper show that, in spite of its having a uterine as opposed to a vaginal character, the uterus masculinus of the langur is not an organ which reacts to prolonged oestrogenic stimulation by sufficient hyperplasia to account for the glandular changes of benign hypertrophy. On the other hand, the present evidence fully confirms the finding (Zuckerman, 1938) that the fibromuscular tissue of the middle lobe (utricular bed) is very sensitive to oestrogenic stimulation, and that the increase in the size of the prostate which results from such stimulation is largely due to changes within this region.

It is therefore of particular interest that a recent study of the pathology of the disease leads Deming & Neumann (1939) to the view that the fibroadenomatous nodules of benign hypertrophy begin as non-glandular growths within the musculature of the upper prostatic urethra—most commonly "near the utricle and below its floor". These two investigators also claim that the epithelial elements within the nodules represent later invasions "from an adjacent duct". Although the nature of the adjacent ducts is not defined, it is perfectly clear that the only ducts which could be implicated are (*a*) glandular offshoots of the uterus masculinus, (*b*) collecting ducts of the prostate,

and conceivably (*c*) dorsal urethral glands above the level at which the collecting ducts enter the urethra. The possibility that the uterus masculinus provides the epithelial elements has already been considered sufficiently both in this and in previous papers, and the view that urethral glands (glands of Albarran) are concerned, a view which to-day has fewer supporters than opponents, is dealt with at length in the clinical literature. The further possibility that collecting ducts of the prostate may be involved has not, however, to our knowledge been considered hitherto. The finding reported in this paper that secreting glands may differentiate within the walls of the terminal parts of collecting ducts which have grown in response to oestrogenic stimulation seems therefore to be particularly significant in this connexion. Given that the fibroadenomata of the enlarged prostate do begin as a result of oestrogenic stimulation within the region of the utricular bed, it is clear that the epithelial elements of the ducts, no less than those provided by the uterus masculinus, would soon be implicated in the process.

Whatever the source of the glandular elements of the growths, Deming and Neumann's view that the disease begins in the fibromuscular tissue of the dorsal urethral wall in the region of the uterus masculinus undoubtedly finds strong experimental support in the experimental observation that the fibromuscular tissue of the region concerned is exceptionally sensitive to oestrogenic stimulation. As has already been pointed out, the topographical relations of this tissue show that it is either wholly or partly derived from the mesoderm of the genital cord, which in the female goes to form the musculature of the uterus. It is therefore necessary to note that the extreme sensitivity of the myometrium to oestrogenic stimulation (see, for example, Barks & Overholser, 1938) may lead to the development of uterine fibromyomata (Lacassagne, 1935; Nelson, 1937; Moricard & Caudoix, 1938).

It may therefore be concluded that the view stated in a previous paper (Zuckerman, 1938)—that "the hyperplasia of a glandular system lying within a stroma which reacts to oestrogenic stimulation as intensely as does the utricular bed of monkeys would undoubtedly produce a structural alteration in the prostate indistinguishable from that of benign enlargement"—is much strengthened by the evidence reported in the present paper. At the same time it must also be pointed out that the pathological changes which result in benign enlargement of the prostate have been regarded, in the experimental work carried out up to now, as being only an intensification of a normal physiological response, and that further experimental studies will very likely show that some additional factor is involved in the aetiology of the disease. At present, however, this possibility can have little influence in discussions on the endocrine treatment of the disorder. Originally male-hormone therapy, which is at present being extensively tried, was advocated as treatment simply because of the presumed association of the disease with "the male climacteric" in which, it was believed, a decline in the production of androgenic hormones occurs. Shortly afterwards this form of treatment was given a more rational

basis in the experimental finding that the changes caused by oestrogenic stimulation in the prostate of laboratory mammals can be related to those which occur spontaneously in benign enlargement in man, and in the knowledge that the effects of oestrogenic stimulation can be counteracted by means of male hormone. The evidence provided by the present paper adds to the rationale of endocrine therapy in the treatment of benign enlargement of the prostate. For not only has it been clearly shown that male hormone can suppress the effects of oestrogenic stimulation, but it has also been demonstrated that while the latter form of stimulation induces growth in that part of the prostate in which the fibroadenomata of benign enlargement begin, the effect of male hormone is to reduce relatively the size of that part of the prostate, and concurrently to stimulate the glandular zone proper, a zone which in benign hypertrophy becomes displaced until it may come to form a false capsule to the much hypertrophied and altered middle lobe.

SUMMARY

1. The effects of castration, of oestrogenic stimulation, of androgenic stimulation, of oestrogenic and androgenic hormone given simultaneously, and of oestrone and progesterone given together on the different zones of the prostate and on the glandular and muscular tissue of the seminal vesicles of immature rhesus monkeys (*Macaca mulatta*) have been analysed quantitatively. It is shown that while oestrogenic hormone decreases relatively the size of the glandular zone and increases markedly that of the "middle lobe" (utricular bed), male hormone has the opposite effect of reducing relatively the latter and increasing greatly the extent of the glandular region. Depending on the proportions in which they are given, both male hormone and progesterone can inhibit the total and partial effects of oestrogenic stimulation on the prostate. In general the same endocrine interactions operate in the case of the seminal vesicles, in which the muscular coat is mainly responsive to oestrogenic stimulation, and the epithelial tissue to androgenic hormone.

2. The general effects of oestrogenic and androgenic stimulation on the prostate of the Hanuman langur, *Presbytis entellus*, are the same as those in the rhesus monkey. In this species, however, the uterus masculinus is uterine and not vaginal in character, and it does not respond to oestrogenic stimulation by squamous metaplasia as does that of the rhesus monkey. On the other hand in neither of the two langurs injected with large doses of oestrone for periods of 483 and 316 days respectively was the glandular hyperplasia which occurred in the uterus masculinus excessive. Glandular hyperplastic changes were, however, also observed in the terminal parts of the collecting ducts of the prostate in the region of the mouth of the uterus masculinus, and also in the terminal parts of the common ejaculatory ducts. Increased stratification of the urethral epithelium occurred on the summit of the crista urethralis.

3. The relation of these findings to the problem of the aetiology and endocrine therapy of benign enlargement of the prostate is discussed.

Our best thanks are due to Mrs M. Griffiths for her help in the statistical analysis of the data. The monkeys were bought with the aid of a grant to S. Z. from the Medical Research Council. The work was also supported by a grant to S. Z. from the Nuffield Medical Committee, Oxford. Our thanks are due too to Dr K. Miescher of the Ciba Co., and to Dr E. Neumann of Messrs Scherings, Ltd. for their kindness in providing the hormones used in the experiments.

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EXPLANATION OF PLATE I

Hanuman langur, *Presbytis entellus* (202), after treatment with oestrone for 483 days.

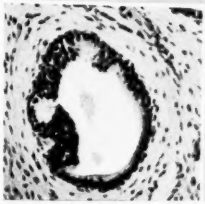
Fig. 1. Normal epithelium over crista urethralis. ×118.

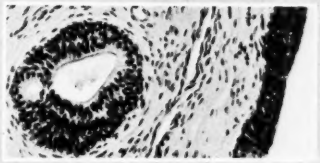
Fig. 2. Epithelium of distal part of common ejaculatory duct showing hyperplasia and a "mucinous" change. ×126.

Figs. 3–5. Hyperplastic epithelium of collecting ducts of prostate, with glandular proliferations in the thickened walls. ×118.

Fig. 1.

Fig. 2.





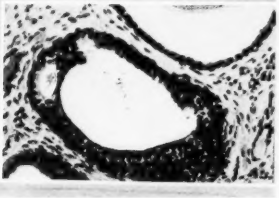


Fig. 3.

Fig. 4.

Fig. 5.

UNIV. OF MICH.

THE EFFECT OF THE COMPLETE REMOVAL OF MUSCLES ON THE GAIT OF THE RABBIT

By D. STEWART

From the Department of Anatomy, University of Manchester

In a previous paper in the *Journal of Anatomy* (1937), the author gave an account of the changes which occur in the gait of the rabbit after the tendons of insertion of the muscles of the calf region had been divided. Six animals were used and the muscles were divided in the following order in the different animals:

Rabbit 11. (*a*) Plantaris, (*b*) soleus, (*c*) inner head of gastrocnemius, (*d*) outer head of gastrocnemius, (*e*) deep flexors.

Rabbits 12 and 14. (*a*) Deep flexors, (*b*) plantaris, (*c*) soleus, (*d*) outer head of gastrocnemius, (*e*) inner head of gastrocnemius.

Rabbit 13. (*a*) Outer head of gastrocnemius, (*b*) inner head of gastrocnemius, (*c*) soleus, (*d*) deep flexors, (*e*) plantaris.

Rabbit 16. (*a*) Soleus, (*b*) deep flexors, (*c*) inner head of gastrocnemius, (*d*) plantaris, (*e*) outer head of gastrocnemius.

Rabbit 18. (*a*) Inner head of gastrocnemius, (*b*) deep flexors, (*c*) plantaris.

Rabbit 14 was a repetition of rabbit 12, as by an oversight, the soleus on one side was not divided.

The investigation was carried out by making slow-motion "cine" films which, when projected on the screen, reduced the apparent speed at which the animal was moving to a quarter of the normal. Examination of the film showed that until three out of the five muscles were divided, no obvious change could be detected in the gait of the animal.

Since the publication of this paper the author has devised a quantitative method of analysis which has been employed for checking the previous results and also for the purpose of analysing a series of experiments in which the corresponding muscles were removed. The method adopted was to draw lines representing the long axis of the different segments of the hind limb and afterwards to measure the variations in the size of the angles made between these segments and the horizontal plane during a complete locomotory cycle: the variations in the size of the angles at the knee and ankle joints were also measured.

From the measurements which were obtained, graphs were plotted, an example of which will be seen in Fig. 1*b*. The unbroken line indicates the variation in the angle of the long axis of the thigh, the broken line that of the leg, and the dotted line that of the foot. The horizontal line of the graph

shows time, as represented by the number of frames of the film, while the vertical represents the size of the angle. At the bottom of the graph, two arrows pointing upwards indicate the moment at which the limb leaves the

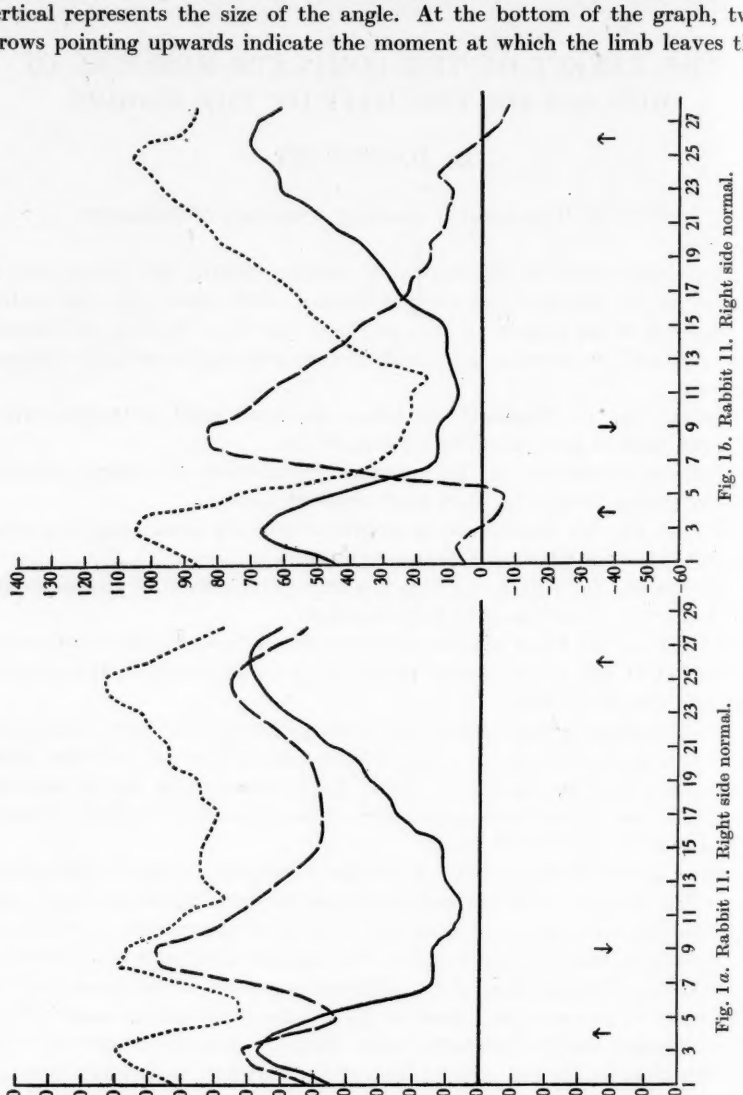


Fig. 1*b*. Rabbit 11. Right side normal.

Fig. 1*a*. Rabbit 11. Right side normal.

ground, the start of the suspense period, while the downward pointing arrow indicates the instant at which it again comes in contact with it, the start of the support period. It is unnecessary to describe the graph in detail, but it

will be observed that the foot and the thigh make their greatest angle at about the commencement of the locomotory cycle, that is, when the foot is leaving the ground, and reach their lowest or most horizontal position when the foot again makes contact with the ground. There is a slight secondary rise about this period. The leg follows an exactly opposite course; it is at its lowest when the foot leaves the ground and at its highest at the beginning of the support

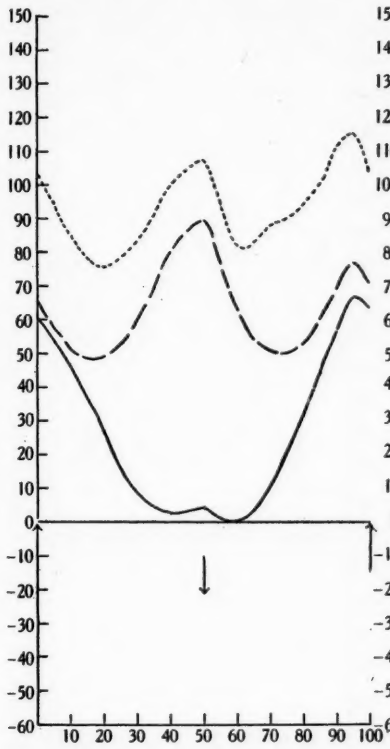


Fig. 2*a*. Normal rabbit average.

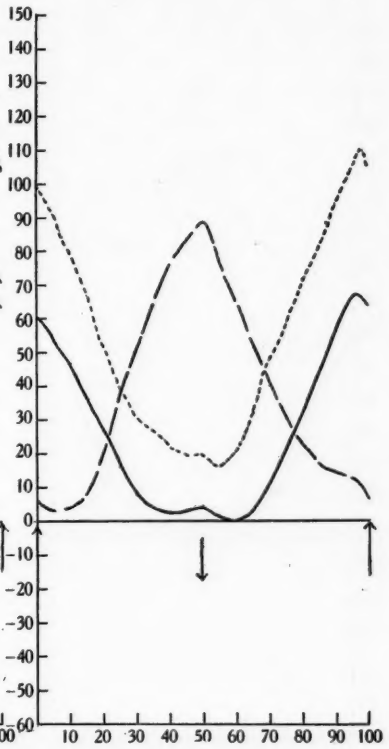


Fig. 2*b*. Normal rabbit average.

period. The second graph (1*a*) shows the variations in size of the angle of the knee and ankle joint. It has not been possible to obtain a satisfactory line to represent the long axis of the body, and in this graph the movements of the hip joint are again represented by the thigh. The broken line indicates the movements of the knee and the dotted line those of the ankle. Here it will be seen that the knee and the ankle are extended at the moment the foot leaves the ground. They flex during the suspense period and afterwards re-extend when the foot comes in contact with the ground. There is a second

period of flexion during the support period, after which the joints again extend.

As the length of the locomotory cycle varies with the rate at which the animal is moving and with individual animals, it follows that the horizontal line of the graph also varies. For purposes of comparison it was desirable to reduce all the graphs to a common base line. This was done without any

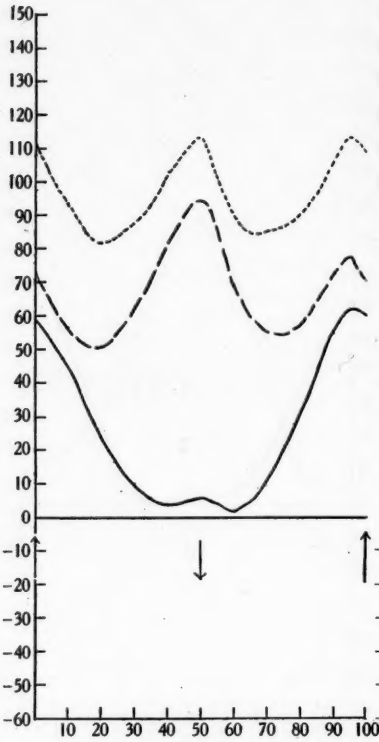


Fig. 3*a*. Rabbit, one muscle cut.

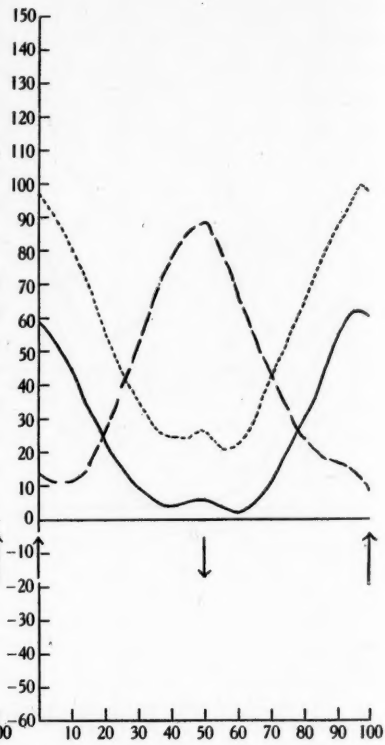


Fig. 3*b*. Rabbit, one muscle cut.

great difficulty by using a slide rule, and all the graphs were reduced to a common length of 10 cm., the first 5 cm. of which represented the suspense period of the cycle and the last 5 cm. the support period. With a common base line, it was easy to make an average graph of the gait of a number of animals.

Average graphs were now made of the films which had been used in the previous analysis and it will be seen that these gave results which were similar

to those obtained from inspection. Fig. 2 shows average graphs of a number of normal rabbits, while Fig. 3 shows graphs from a number of rabbits each of which had one muscle divided at its tendon. It will be clearly seen that there is no alteration in the shape of the graphs. This also applies to Fig. 4 in which the graphs were made from the same animals after two muscles had been cut, and again the change in the graph is negligible. After three muscles have been

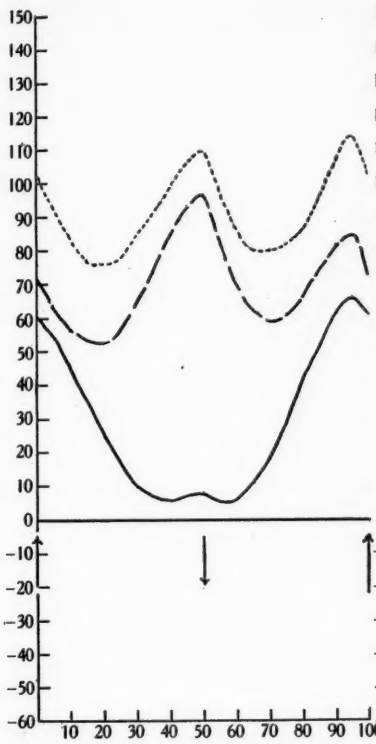


Fig. 4*a*. Rabbit, two muscles cut.

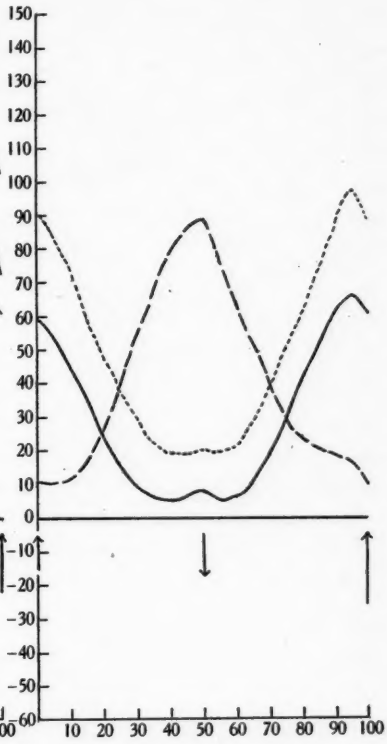


Fig. 4*b*. Rabbit, two muscles cut.

divided (Fig. 5), it will be noticed that there is a distinct increase in the amount of flexion of the ankle during the support period. This is due to the raising of the foot being delayed. It is certainly not caused by any alteration in the movement of the leg, as this graph is unaltered. After four muscles have been divided (Fig. 6), the delay in raising the foot is slightly increased and the amount of flexion of the ankle is also greater. The final graph of this series (Fig. 7) shows the changes which take place in the gait after section of the

tendons of all the calf muscles, namely, both heads of gastrocnemius, soleus, plantaris, and the deep flexors, and here a very marked change has taken place. There is an increase of both suspense period and support period flexion of the ankle joint and the time of this flexion is prolonged. This is clearly due to the position taken up by the foot. Inspection of this graph reveals that towards the end of the suspense period the foot is tilted beyond the horizontal.

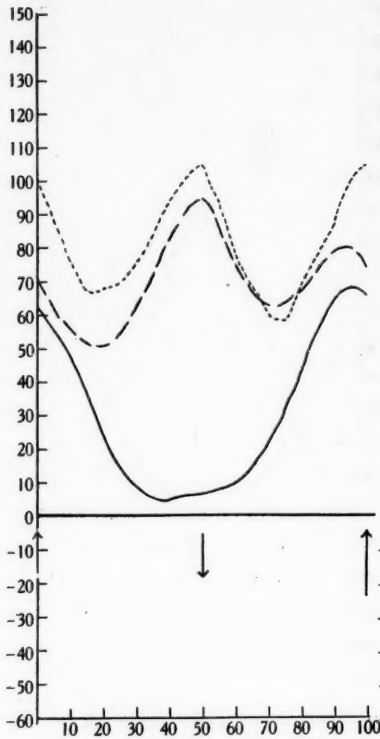


Fig. 5*a*. Rabbit, three muscles cut.

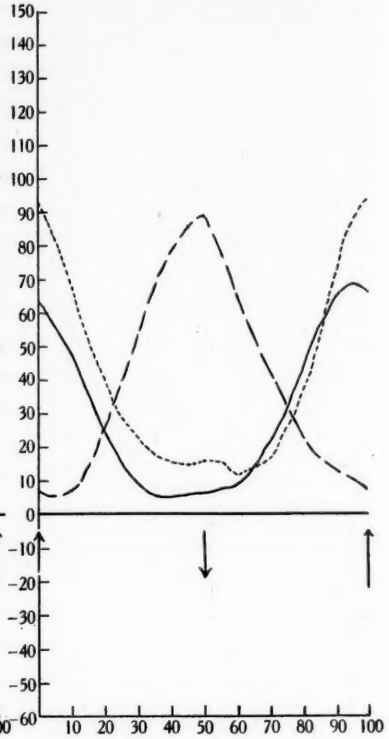


Fig. 5*b*. Rabbit, three muscles cut.

It therefore reaches the ground by making contact with the heel, giving for the first time complete plantigrade gait such as seen normally only in man. These graphs therefore confirm the results which were recorded in the original paper.

In the next stage of the investigation certain of the calf muscles were completely removed. The experiments were done with a series of ten animals;

in each case two muscles of the group were removed, differing from animal to animal, namely,

Outer head of gastrocnemius + inner head of gastrocnemius.
Outer head of gastrocnemius + plantaris.
Outer head of gastrocnemius + soleus.
Outer head of gastrocnemius + deep flexors.

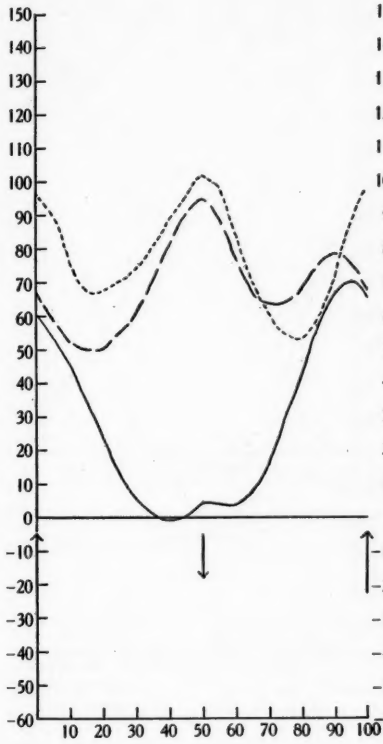


Fig. 6*a*. Rabbit, four muscles cut.

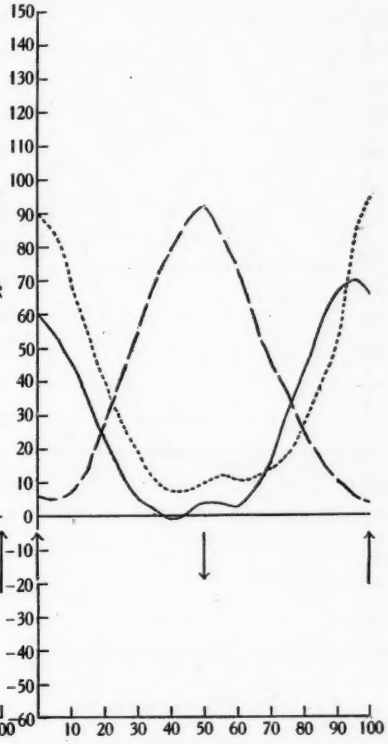


Fig. 6*b*. Rabbit, four muscles cut.

Inner head of gastrocnemius + plantaris.
Inner head of gastrocnemius + soleus.
Inner head of gastrocnemius + deep flexors.
Plantaris + soleus.
Plantaris + deep flexors.
Soleus + deep flexors.

Fig. 8 shows average graphs from this series of experiments and is for comparison with the graphs in the former series where the tendons of insertion only had been sectioned. If these graphs are compared with Fig. 4 showing the results of experiments in which the muscles only had their tendons of insertion divided, it will now be seen that the actual removal of the muscles

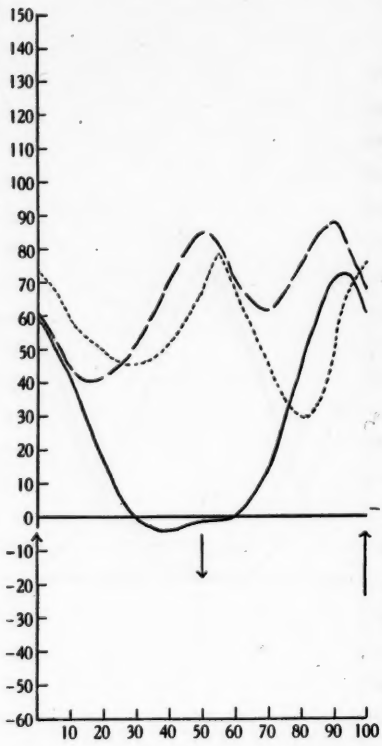


Fig. 7*a*. Rabbit, all muscles cut.

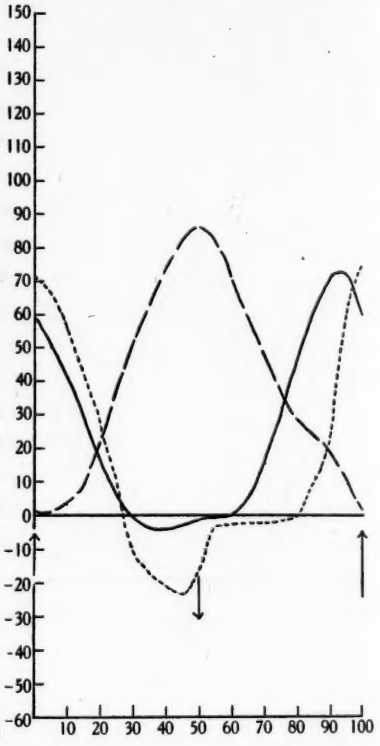


Fig. 7*b*. Rabbit, all muscles cut.

makes a considerable difference to the gait. The extension and flexion of the ankle joint are at a lower level and the foot comes nearer to the horizontal. It is only after four tendons have been divided that the graphs more or less correspond, but even here, the period of support flexion is more prolonged in the animals in which the two muscles have been removed.

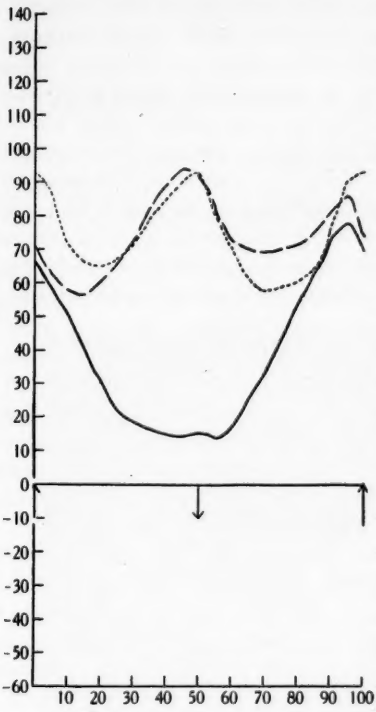


Fig. 8*a*. Rabbit, average, two muscles removed.

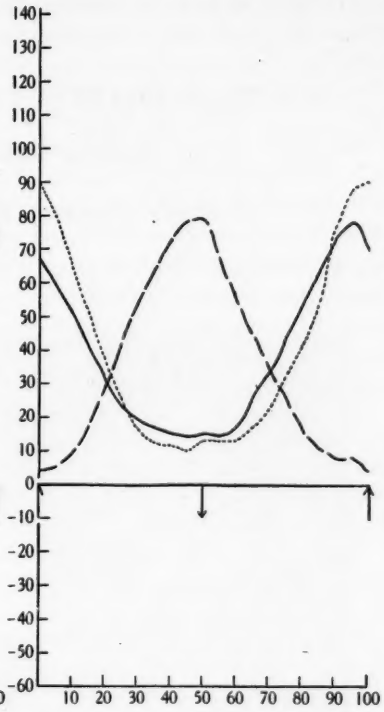


Fig. 8*b*. Rabbit, average, two muscles removed.

SUMMARY

1. A graphical method of analysing gait is described.

2. It is confirmed that division of the tendons of insertion of two muscles in the calf region in the rabbit does not alter the gait of the animal.

3. It is shown that the actual removal of two muscles from the calf region has as much effect upon the gait as the division of the tendons of insertion of four muscles.

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PLACENTA PRAEVIA IN A RHESUS MONKEY

By A. PALMER, *Rockefeller Research Fellow*

From the Department of Human Anatomy, Oxford

Placenta praevia occurs in about 0·1 %, and the central type no more frequently than in 0·02 % of human births (Williams, 1936). To my knowledge no variety of the condition has ever been reported in subhuman Primates, and for that reason an account of central placenta praevia in a rhesus monkey (*Macaca mulatta*) is presented in the following paper.

The normal placenta in the rhesus monkey consists of two disks. The larger disk constitutes the primary placenta and the umbilical cord is invariably attached to it. The secondary and smaller disk, which only rarely is completely absent, is supplied with blood through vessels that pass along the membranous chorion separating it from the primary placenta (Wislocki, 1929).

Case history. A female rhesus monkey (DB 1081/28) that had been in the Gardens of the Zoological Society of London 2 years and 3 months, died from secondary anaemia following a profuse vaginal haemorrhage on the night of 30 November 1928. Keepers reported that she had been perfectly well during the day of 30 November. There were blood clots in the vagina and marked pallor of all mucous membranes. The animal appeared to have been in the first stage of labour and to have died of haemorrhage from a placenta praevia. The fact that the animal was pregnant had not been recognized, nor was there any history of a previous pregnancy. The post-mortem examination (which was made by Dr S. Zuckerman) revealed no evidence of acute infection. All organs were pale and "washed out". Pathological findings were otherwise restricted entirely to the uterus and its contents. An apparently full-term uterus, free, except for its normal pelvic attachments, was found filling the abdomen.

Description of the specimen (after fixation in formalin and preservation in alcohol for 10 years). The specimen consists of the entire uterus and the fleshy birth canal. The outer surface of the uterus is uniformly smooth except for a somewhat increased vascularity along the left side of the lower uterine segment. The specimen was opened by an anterior longitudinal incision. The cervix is patulous with the placenta presenting. A full-term female foetus is present in the LOA position. It has a sitting height of 145 mm. and weighs 247 g. The umbilical cord measures 16 cm. in length. Other measurements are as follows: greatest uterine width, 10·2 cm.; thickness of uterine wall in the fundus 3 mm.; distance between top of fundus and internal cervical os 0·4 cm.; length of cervical canal 2 cm.; and length of vagina approximately 5·5 cm.

The placenta has the form usual to rhesus monkeys, and the umbilical cord is inserted slightly eccentrically into the larger of the two disks. The superior margin of this disk is attached 4 cm. above the internal os along the left latero-posterior surface of the uterus, and nearly half of it has herniated into the cervical canal, having been torn from what had apparently been its firm attachment to a closed internal os. At the internal os on the right side its inferior margin joins the smaller disk, which is attached to the right side of the lower half of the uterine wall above the internal os. The inferior margin of the smaller disk is also partially separated and it lies in the upper part of the cervical canal. Non-vascular membrane and superficial vessels are the only communicating structures between the two disks.

Text-fig. 1. Central placenta praevia in rhesus monkey.

The larger disk measures 6×11 cm., and the smaller one 5×9 cm. The part of the larger disk lying in the cervical canal measures 4×5 cm. The average thickness of the placenta is slightly less than 1 cm., and in all it weighs 75 g.

Microscopic examination. All tissues are slightly infiltrated with white blood cells. The lower part of the cervical canal is otherwise normal in appearance, and the line of transition between the vaginal epithelium and the cylindrical cervical epithelium occurs in the middle third of the canal. The cervical stroma contains a few cervical glands which are distended with mucus. The mucosa of the upper third of the canal, although it is unrelated to the site of placental implantation, is deeply plicated (Pl. I, fig. 1), and its superficial stroma is more cellular than usual (Pl. I, fig. 2). The cells are polygonal and have fairly distinct cell walls, and this fact, coupled with their network arrangement, suggests that this region of cervical stroma has undergone a modified

decidual reaction. It may be noted that swollen decidual cells have been described in the human cervix in cases of placenta praevia (Morton, 1937).

Several large and thick-walled vessels are to be seen in the myometrium of the placental site (Pl. I, fig. 3). Their walls consist almost entirely of many layers of swollen epitheloid cells which appear to have undergone a decidual change (Pl. I, fig. 4). The large amount of cytoplasm in these cells appears to distinguish them from the supposed endothelial proliferations which occur in early pregnancy in the uterine vessels of the macaque (Fig. 47 in Wislocki & Streeter, 1938). The preparation of the material appears to have caused dissolution of cytoplasmic granules and a shrinkage of cells within a fibrous matrix. It is probable that this vascular reaction is equivalent to the alterations which occur in the vessels of the human uterus in preparation for their postpartum closure.

It may be noted that cells similar to those described in the preceding paragraph occur in the walls of arteriovenous anastomoses (Schumacher, 1938). It is generally believed that these cells are differentiated within the media, and that in spite of their epithelial appearance, they are not derived from the endothelium as Wislocki & Streeter suggest they are in the vessels of the pregnant uterus. This view depends upon the fact that, in arteriovenous anastomoses a distinct and continuous layer of differentiated epithelioid cells covers the large cells to which a chemical or hormonic function, in addition to a mechanical one, has been ascribed.

SUMMARY

A case of central placenta praevia in a rhesus monkey is described. Among the points of interest noted are a modified decidual reaction in the cervical stroma and the presence of thick-walled vessels in the myometrium of the site of placental implantation.

My best thanks are due to Prof. W. E. Le Gros Clark for his generosity in furnishing facilities for working in his department, and to Dr S. Zuckerman for permission to describe the specimen.

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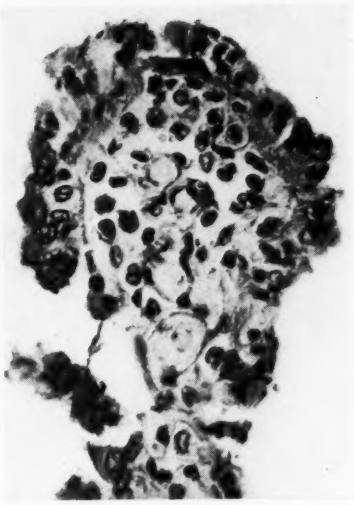
Fig. 1.



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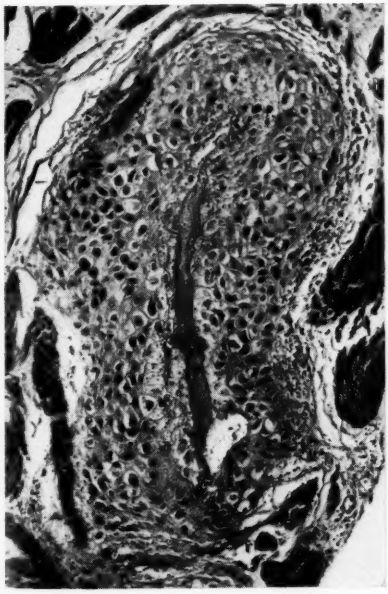

Fig. 3.

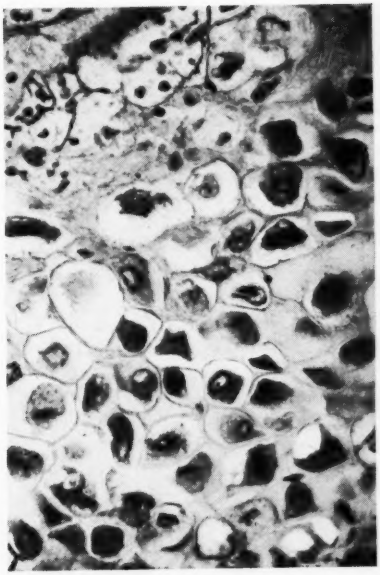

Fig. 4.

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EXPLANATION OF PLATE I

Fig. 1. Photomicrograph showing plication of cervical mucosa in upper third of cervical canal. ×60.

Fig. 2. Photomicrograph showing modified decidual reaction in superficial stroma of cervix; enlarged from Fig. 1. ×450.

Fig. 3. Photomicrograph showing occluded thick-walled maternal vessel in the myometrium of the placental site. ×75.

Fig. 4. Photomicrograph showing decidual-like cells in wall of vessel. ×450.

THE CORACOID PROCESS IN THE PRIMATES

By C. P. MARTIN and H. D. O'BRIEN
Department of Anatomy, McGill University, Montreal, Canada

The comparative anatomy of the human scapula has been worked out in considerable detail, but in the course of this work one part of the bone—the coracoid process—has been almost completely neglected, and the authors of the standard text-books on the subject generally content themselves with remarking that it displays a great range of individual variation even within the same species. Possibly this almost universal inconstancy in the size and form of the process discouraged investigation and led to an idea that in the midst of such constant individual differences it would be vain to search for interspecific differences. Nevertheless, the human coracoid process differs constantly from that of other animals in some features, for although it varies greatly in these peculiarly human characteristics, yet the range of variation found within the human race does not overlap that found in other Primates or overlaps it only to a very slight extent.

The peculiar characteristics of the human coracoid are best displayed when the scapula is viewed from the lateral aspect with the centre of the glenoid cavity opposite the observer's eye, and in Fig. 1 the outlines of the glenoid region of two scapulae, one that of an adult female chimpanzee and the other a typical human specimen, are shown as seen from this aspect. It will be noticed that in the chimpanzee bone the root of the coracoid process is directed upwards and slightly forwards (much more upwards than forwards), and from the upper end of the root the body of the process runs downwards, forwards and laterally, though of course in this aspect of the bone the lateral component is not visible. The root and body of the process meet at an acute angle or at most in some cases at a right angle. In the human bone, on the other hand, the root of the process runs forwards and upwards (more forwards than upwards), and the body of the process is directed forwards, very slightly downwards, and laterally, and the root and body of the process meet at a widely obtuse angle. When the long axis of the glenoid cavity is used as a line of reference, therefore, the coracoid process differs considerably in the two bones.

In making these drawings the surface of the glenoid cavity was used as a plane of reference and was placed parallel to the glass of the dioptograph. But is the plane of the glenoid cavity a reliable reference plane or does it vary in the two species? In the chimpanzee and other great apes the long axis of the glenoid cavity and the vertebral border of the scapula, if produced, meet at approximately an angle of 30°, but in man these two lines are parallel or meet at a very acute angle (Martin, 1928). This suggests that in the chimpanzee the

glenoid looks more cranialwards than in man, and, if so, in these drawings the human coracoid has been viewed from a lower point than the chimpanzee coracoid, and this may account at least to some extent for their different appearances. But it seems more likely that the differences between man and the great apes in respect of the vertebro-glenoid angle, or the angle at which the vertebral border of the scapula and the long axis of the glenoid cavity meet, are due to changes in the vertebral border of the bone than to alterations in the direction of the glenoid cavity. For the spino-scapular angle or the angle between the base of the spine and the vertebral border of the scapula varies proportionately to the gleno-vertebral angle; in the great apes it is somewhere near 50°, in man it is almost a right angle. The plane of the glenoid cavity

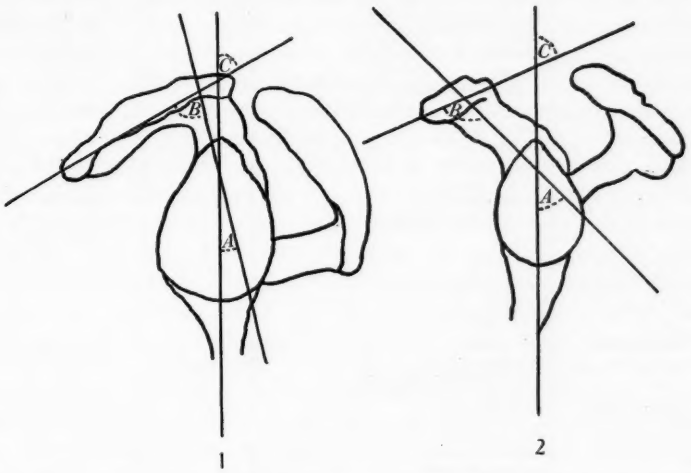

Fig. 1. Dioptograph tracings of glenoid region of scapula of (1) chimpanzee and (2) man. The various axes are shown on the drawings and the angles between them are designated: *A*, lower coracoid angle; *B*, upper coracoid angle; *C*, final coracoid angle.

therefore appears to be almost constant in reference to the base of the spinous process. If a human and a chimpanzee scapula are looked at from behind and placed with their axillary borders parallel, the glenoid cavity of the chimpanzee looks more cranialwards than does that of the human specimen, or in other words the gleno-axillary angle in the chimpanzee is considerably larger, viz. by about 17°, than in man. But here again the spino-axillary angle—that is, the angle formed by the axillary border and the base of the spine—is about 17° less in the chimpanzee than in man (Schultz, 1930). The plane of the glenoid cavity therefore bears a fairly constant relationship to the base of the spinous process, and in fact the relationship of the spinous process to the plane of the glenoid cavity appears to be one of the most constant features in the scapula. In Table I the values obtained for the gleno-spinal angle in a comprehensive series

of Primates and also in a fairly extensive representative series of other mammals are shown. In the orang the angle sometimes fell as low as 60°. This is largely due to the fact that in this species the vertebral end of the scapular spine often turns abruptly upwards. Schultz's dioptograph drawings of the orang scapula in the paper referred to above strikingly illustrate this fact, and as this upturning places the root of the spine at a considerably higher level than in other species the gleno-spinal angle must be correspondingly reduced.

Table I. *The gleno-spinal angle*

	Species	No. of bones measured	Mean values and ranges (in degrees)
PRIMATES	*Homo*	20	92 (83–103)
	Gorilla	19	87 (82–93)
	Pan	6	91·5 (88–99)
	Pongo	4	75 (60–90)
	Hylobates	6	91·5 (85–102)
	Macaca	3	102 (90–110)
	Cercopithecus aethiops	2	103·5 (102–105)
	Papio spp.	2	92·5 (90–95)
	Cebus frontales	1	85
	Alouatta	1	80
	Hapale jacchus	2	87 (85·5–88)
	Saimiri sciurea	2	93 (87–99)
	Tarsius spectrum	2	101 (101–101)
	Lemur fulvus nigrifrons	2	97·5 (93·5–105·5)
	Loris	2	87·5 (84·5–90·5)
UNGULATA	*Tapirus* sp.	2	105·5 (104–107)
	Dicotyles tajacu	2	102 (100–104)
	Ovis spp.	1	115
	Equus spp.	1	101
CARNIVORA	*Felis pardus*	2	104 (104–104)
	Felis domesticus	2	112 (110–114)
	Canis familiaris (mastiff)	1	109
CHIROPTERA	*Desmodus rufus*	2	117·5 (117–118)
RODENTIA	*Lepus cuniculus*	2	111·5 (106–117)
	Hyrax capensis	2	106·5 (101–112)
	Dasyprocta agouti	2	121·5 (119–124)
	Hystrix cristata	2	110·5 (110–111)
EDENTATA	*Choloepus didactylus*	2	113·5 (111–116)
MARSUPIALIA	*Macropus giganteus*	1	86

Within the Primates the angle varies from 60 to 110°—a variation of 50°. But if we exclude the orang, in which for the reasons stated above the angle is exceptionally low, we find that it varies between 82 and 110°—a variation of 28°. According to Martin (1928) the scapulo-spinal angle within the higher Primates varies between 32 and 88°—a variation of 56°—and, as he only gives mean values for the angle in different species, the absolute range of variation almost certainly exceeds this figure. According to Schultz (1930) the gleno-axillary angle of the scapula varies within the Primates between 11 and 58°—a variation of 47°. But the gleno-spinal angle in all Primates varies little from a right angle. In quadrupeds it is about 110°, and its higher value in them seems to be mainly due to a protrusion of the upper end of the glenoid. In the one marsupial measured the angle fell to 86°. We therefore concluded that the plane

of the glenoid was as reliable a reference plane as could be obtained, and that the two drawings of the coracoid process in man and the chimpanzee shown in Fig. 1 are strictly comparable.

An endeavour to measure these differences in the process between man and the other animals was then undertaken. The scapulae were set up in the dioptograph with the plane of the glenoid parallel to the glass of the dioptograph, and the outlines of the glenoid cavity, of the coracoid process, and in some cases of the spinous process and acromion were traced. Great care is necessary in orientating the bones, as slight variations in position produce marked variations in the angles. Our method was to lay a ruler on its edge first lengthwise on the glenoid cavity and then transversely and in both cases make sure that the edge of the ruler was parallel with the glass of the diptograph. On these tracings the long axis of the glenoid cavity, the axis of the root of the coracoid and the axis of the body of the coracoid were then drawn, and these different axes are shown on the bones in Fig. 1. It is not easy to determine the long axis of the glenoid cavity with exactitude, and in an endeavour to facilitate the determination of this axis the spine of the scapula was also traced in many bones and the angle between the estimated long axis and the axis of the spine was measured, but this method was not found to be of much assistance, and thereafter the axis was drawn visually with as much accuracy as was possible. In the case of the human bones especially, the axis of the body of the coracoid process also presented difficulties, for the tip of the process is often slightly turned down, making the axis a curve. In human bones therefore we drew the axes of the glenoid cavity and of the body of the coracoid on the bones, traced them on the dioptograph drawings, and drew a straight line corresponding as closely as possible to each axis. But it is not possible to make this method absolutely precise, and allowance must be made for experimental error. Further, in dealing with the bones of very small animals such as *Tarsius* or the lemurs, it was concluded that more accurate results were obtained by mounting the bones in the dioptograph as before and then tracing the various axes with the dioptograph while viewing the actual bones than in making dioptograph tracings of the bones and subsequently drawing the axes on them. In the latter case there are two occasions for error, one in making the tracing and the other in drawing the axes on the tracing, and in such small bones a minute error on either occasion would seriously distort the results; but in the former case there is only one opportunity for error.

The angles between these axes were then measured and these angles are shown in Fig. 1 and designated *A*, *B* and *C* respectively. *A*, the angle between the long axis of the glenoid cavity and the axis of the root of the coracoid process, we call the lower coracoid angle; *B*, the angle between the axis of the root and the body of the coracoid process, we call the upper coracoid angle; and *C*, the angle between the produced long axis of the glenoid cavity and the axis of the body of the coracoid process, we call the final coracoid angle. Obviously *A* plus *C* must equal *B*. The results of measuring these angles in

Table II. *The coracoid angles*

Species	No. of bones measured	Mean values and ranges (in degrees)		
		Lower coracoid angle	Upper coracoid angle	Final coracoid angle
Homo	35	44·7 (30–65)	113·3 (100–133·5)	68·5 (50–95)
Gorilla	32	28·2 (8–40)	75·8 (61–91·5)	48·2 (34–67·5)
Pan	31	35·5 (17–68)	70·6 (56–89·5)	37·3 (16–53)
Pongo	8	39·4 (23–53)	79·9 (71–87·5)	40·6 (29–51)
Hylobates	4	46·5 (37–57)	90 (87–96)	43·5 (33–52)
Macaca mulatta	27	47 (31–64)	88 (74–108·5)	42 (30–62)
Macaca sinica	2	62 (57–67)	97 (91–103)	35 (34–36)
Macaca fuscata	2	45 (35–55)	97 (90–104)	52 (49–55)
Cercocebus torquatus	8	46·6 (29–62)	83 (80·5–107)	44·5 (33–54)
Cercopithecus aethiops	2	37·7 (37·5–38)	91 (90–92)	53·2 (52·5–54)
Presbytis priamus	2	47 (44–50)	89 (88–90)	42 (38–46)
Papio spp.	14	40 (23–62)	82·7 (69–101·5)	41 (30–58·5)
Cebus frontales	2	56 (40–72)	80 (65–95)	24 (23–25)
Ateles geoffroyi	2	32 (30–34)	70·5 (65–74)	37·5 (35–40)
Saimiri sciurea	2	57·5 (49–66)	84·5 (79–90)	26·5 (23–30)
Hapale jacchus	2	44·5 (43–46)	84·5 (84–85)	40·5 (39–42)
Tarsius spectrum	2	54 (45–63)	105·5 (103–108)	52 (46–58)
Perodicticus potto	1	54	91	37
Daubentonia madagascariensis	2	39 (34–44)	89 (85–93)	50·5 (50–51)
Lorisidae spp.	3	37·2 (20–52)	83·2 (64–95)	46 (44–50)
Lemur fulvus nigrifrons	2	57 (52–62)	98 (90–106)	41 (38–44)

various scapulae are set out in Table II. These figures show that the coracoid process varies within wide limits even in the same species. In the case of the lower coracoid angle the ranges of variation found in almost all species overlap those found in man, but the mean value found in human bones is above that of the three great anthropoid apes. In the upper coracoid angle the mean value in human bones is far above that found in any other species, but the range of variation found in man is overlapped by that found in *Macaca*, *Cercocebus*, *Papio*, *Tarsius* and *Lemur*, but not by the three anthropoid apes. In the final coracoid angle the mean value in human bones is again far higher than in any other species, but the human range of variation is overlapped by that in the three anthropoid apes and in *Hylobates*, *Macacus*, *Cercocebus*, *Cercopithecus*, *Papio*, *Tarsius* and *Daubentonia*. Of course it must be remembered that in the case of some species only a single individual was examined, and if more were available the range of variation of the different angles might be found to have wider limits. But even allowing for this possibility the human coracoid appears to possess peculiar characteristics which distinguish it from that of all other Primates. In Fig. 2 these results are shown graphically for those species in which a number of bones were measured.

On account of the ordinates of the graph indicating 5°, it appears as if in respect of the upper coracoid angle there was some overlap between man and other Primates, but this is not actually the case. No definite sexual or age variations in the process were discovered, nor was any difference found to exist between the bones of the left and of the right side. Figs. 3 and 4 show the coracoid process in various species.

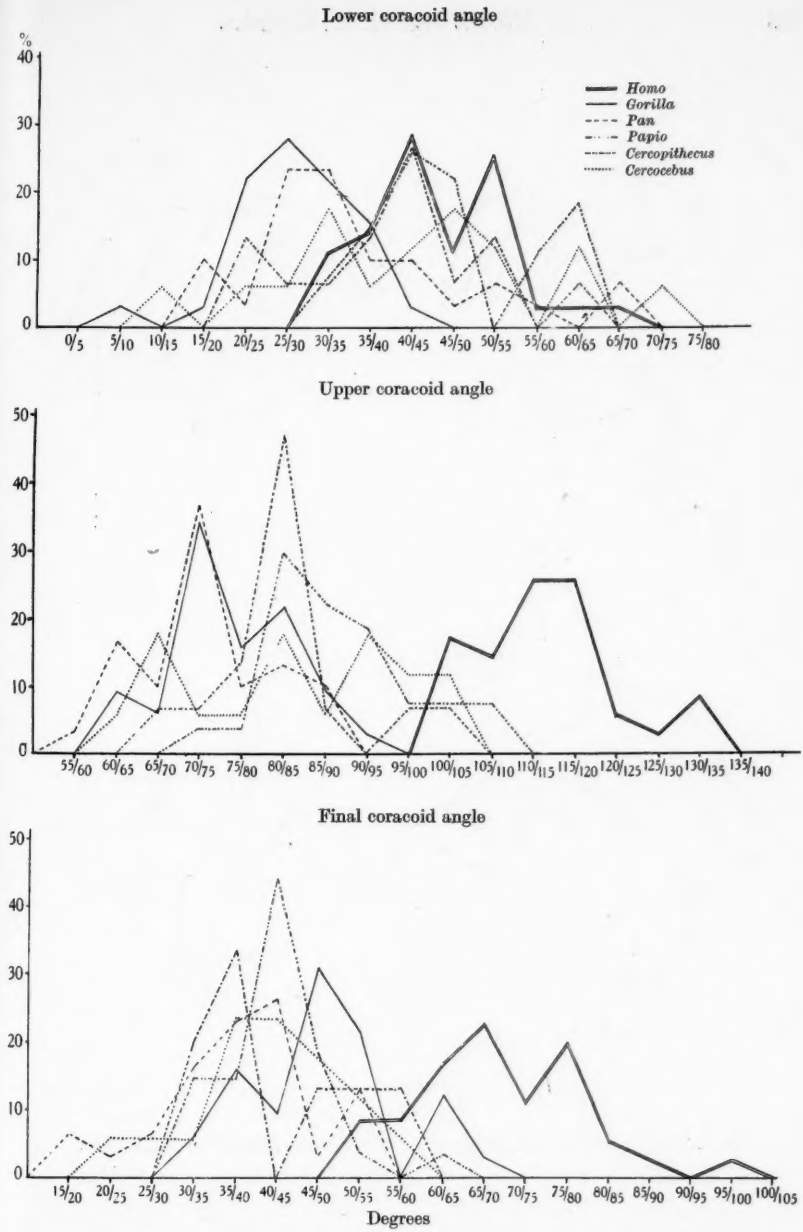

Fig. 2. Graphs showing distribution of the various coracoid angles. Upper graph: lower angle. Middle graph: upper angle. Lower graph: final angle. Ordinates represent percentage of total number of bones having angles lying in the 5° groups marked on the abscissae.

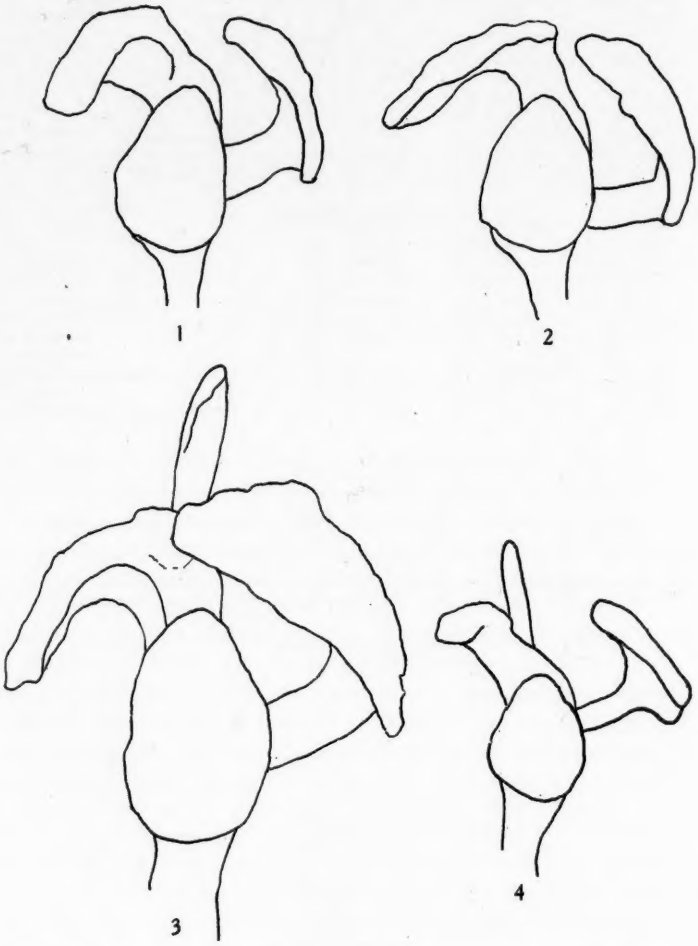

Fig. 3. The coracoid process of (1) orang, (2) chimpanzee, (3) gorilla and (4) man.

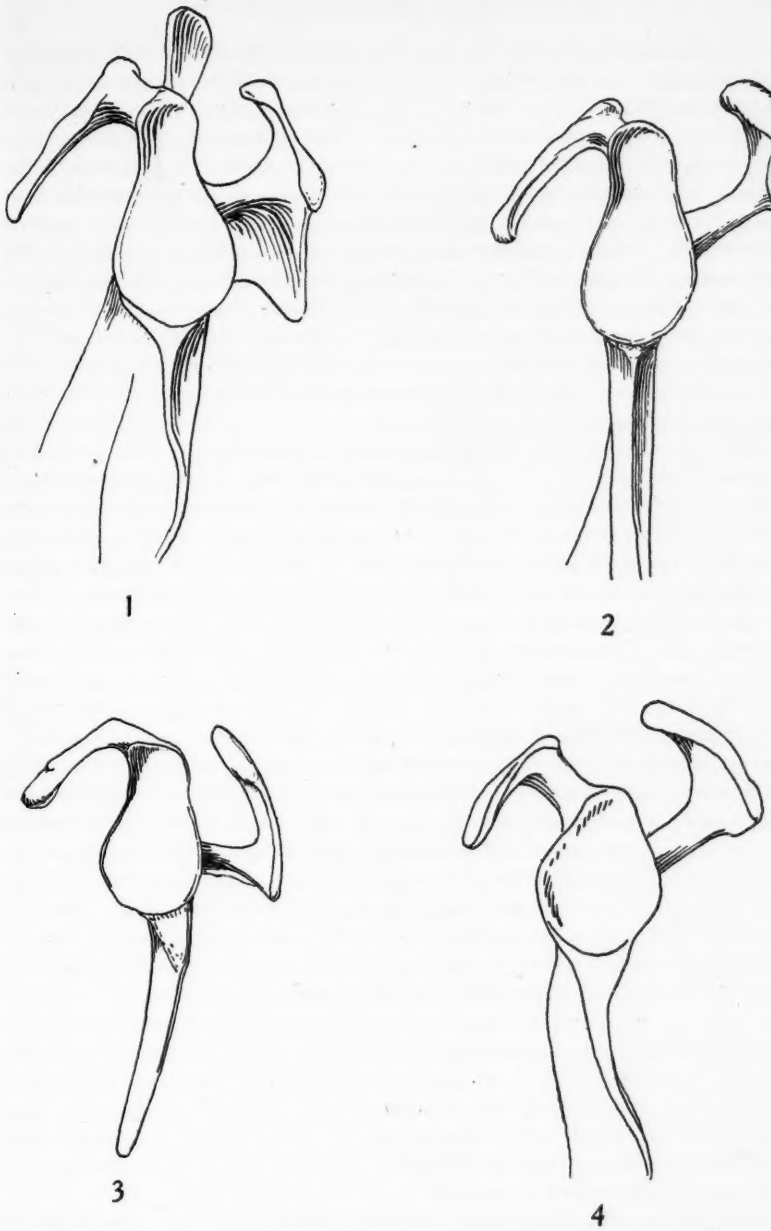

Fig. 4. The coracoid process of (1) *Lemur fulvus nigrifrons* (× 4), (2) *Loris gracilis* (× 4), (3) *Tarsius spectrum* (× 7) and (4) *Hylobates mülleri* (× 2).

Having ascertained that the human coracoid process is distinguished by certain peculiarities, it is rather more difficult to explain the purpose and object of these modifications, but that they have arisen in response to a functional need seems to be a reasonable assumption. The process serves two important purposes: first, it is the main hold by which the clavicle is attached to the scapula, and secondly it, along with the acromion and coraco-acromial ligament, forms the arch above the glenoid cavity. It also serves as a point of attachment for certain muscles. Its most important function appears to be the provision of a strong hold for the ligaments connecting it with the clavicle and in assisting in abduction of the arm, for in almost all animals in which the clavicle has disappeared and the power of abducting the forelimb is lost the coracoid process has been reduced to a mere knob hardly distinguishable from the rest of the bone. Its use as an attachment for muscles seems therefore to be of minor importance.

The process is attached to the clavicle by the powerful conoid and trapezoid ligaments and by this means is kept in constant relationship to the latter bone. Unfortunately in the great majority of the articulated skeletons in our museums and universities this attachment is overlooked and the coracoid is allowed to sink down far below the level of the clavicle, so that the whole shoulder region is somewhat lower than it would be in a living subject. This error arises owing to the scapula being placed too close to the chest wall and its inferior angle probably placed a little too far back. If the scapula is well padded out from the chest wall and braced up, the coracoid comes into its correct position and the whole shoulder region is slightly raised. In articulating skeletons, better and more life-like results would be obtained if the coracoid was joined to the clavicle by a short piece of wire passing through a pad about half an inch thick.

If a comparison be made between man and any of the apes in regard to the shape of the upper end of the body, one is at once struck by the distinctness of the neck and the squareness of the shoulders in the former. In all the apes the shoulders are high and the head is sunk down between the shoulders so that the neck appears to be short and poorly marked off from the thorax. In other words, in all the apes the shoulders are in the "shrugged" position. Schultz (1931) gave illustrations of the shape of the body in man and the anthropoid apes, accurately constructed from measurements, which strikingly bring out this fact. In the lower pronograde apes this position enables the forelimb to reach forwards to its maximum extent; in the higher brachiating apes the shoulder is of course pulled constantly into the shrugged position, and there is in general no other constant force tending to make the shoulder adopt any other position when at rest, for the brachiating apes when not suspended by their forelimbs are generally leaning forwards on their knuckles, which also tends to push the shoulder region upwards.

But once man had adopted a bipedal upright attitude the whole weight of the upper limb and shoulder region became suspended from the head and neck region and slightly from the upper end of the thorax, and the shoulder apparently

gradually sank. The neck became more distinct, the shoulders became either square or even slightly sloping. The shoulder region is probably still sinking in the ontogeny of individual men, as is indicated by the large number of cases in which a cervical rib does not cause any symptoms of pressure on the lower trunk of the brachial plexus until middle life is reached. As the shoulder region sank, the scapula apparently was slightly rotated, its inferior angle passing inwards towards the spinal column. If we shrug our shoulders the scapula rotates slightly with the inferior angle passing outwards and on allowing our shoulders to sink again the scapula rotates in the opposite direction. The inward movement of the inferior angle of the scapula, as the shoulders sank, was probably due to the pull exerted by the rhomboid and levator scapulae muscles. It is also likely that the inferior angle of the scapula was pushed directly backwards for the circumference of the thorax increases downwards at least in its upper half, and moreover in man this region of the thoracic wall was pulled back by the tension of the ilio-costalis and longissimus muscles in maintaining the body upright. The pull of the ilio-costalis gave rise to the angle so characteristic of human ribs and also produced the deep paravertebral gutter on the inner aspect of the body wall which is so well developed in man. All those modifications would tend to displace the inferior angle of the scapula backwards.

Meanwhile the coracoid was firmly held by the conoid and trapezoid ligaments or perhaps it would be more accurate to say that the coracoid had to retain its close relationship with the clavicle in order to maintain the stability of the claviculo-scapular articulation. The result was an opening out of the upper coracoid angle.

If the skeleton of the thorax in man be compared with that of one of the great apes, one of the most conspicuous differences to be noticed is the great relative increase in the breadth of the thorax in man, especially in the region of the second, third and fourth ribs. Schultz (1930), referring to this difference between the thorax of man and that of the apes, states that the broadening of the upper end is already present to a large extent in the gibbon and gives figures illustrating this fact. But his statement is slightly misleading, for this increase in the breadth of the upper end of the thorax is present in the gibbon to a higher degree than in the three larger anthropoid apes. This widening of the thorax pushed the scapula round on to the back, so that in man the glenoid cavity looks more laterally than in the anthropoid apes; and in the latter it looks more ventrally. But as the scapula was pushed back and the lateral border was turned more laterally, the lateral end of the clavicle was carried back with it and in man the shoulders are not only lower than in the apes but farther back also. Consequently the angle between the clavicle and the upper border of the scapula, when viewed from above, has not become more acute in man than in the apes but rather appears to have become slightly more obtuse. Unfortunately we have only been able to examine skeletal material for this feature and in such material errors in correct articulation of the bones provide abundant pitfalls. But the body of the coracoid process in man is directed more

laterally relative to the plane of the glenoid cavity than it is in the anthropoid apes. Fig. 5 shows the coracoid process of an adult chimpanzee and of a typical human scapula viewed from below, and it is evident that in the latter the body of the process forms a larger angle with the plane of the glenoid. The widening

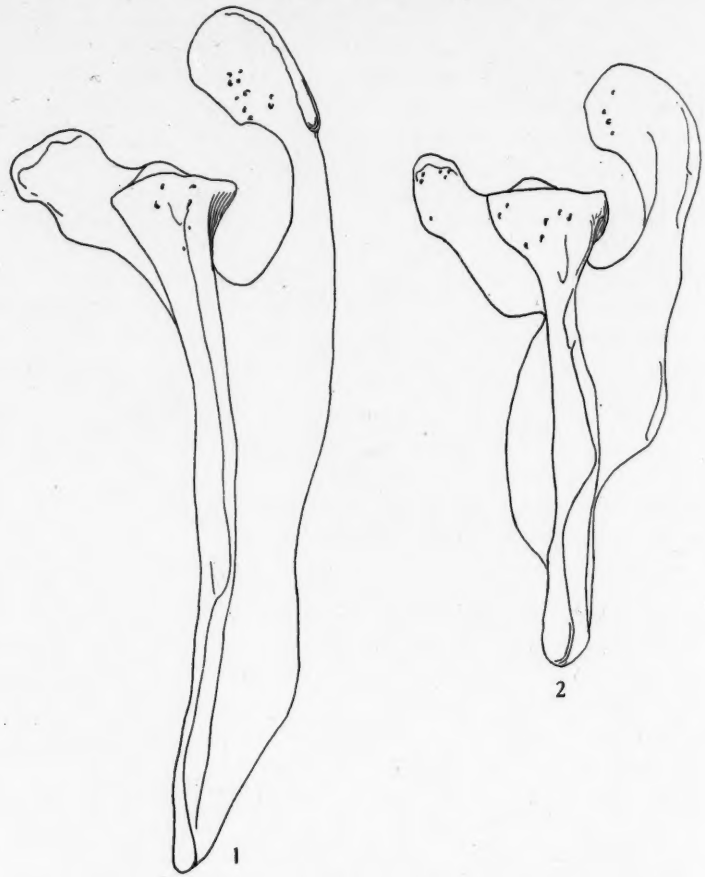

Fig. 5. The coracoid process of (1) chimpanzee and (2) man, seen from below. Dioptograph tracings.

of the angle between the clavicle and the upper border of the scapula in man also explains the forward direction of the root of the coracoid process in human bones.

The absolute length of the coracoid process, particularly of the body of the process, varies greatly between different Primates. As the process seems to be

most directly related to the clavicle an index was worked out in which the length of the body of the process was expressed as a percentage of the maximum clavicular length and the values obtained for this coraco-clavicular index are shown in Table III. The index varies over a wide range. It is highest in

Table III. *The coraco-clavicular index*

Species	No. of bones measured	Mean values and ranges (in degrees)
Homo	14	31·5 (27–36·8)
Gorilla	22	39 (29–46·6)
Pan	25	34·4 (28·2–39·9)
Pongo	6	21·9 (20·5–23·2)
Hylobates sp.	3	21·5 (20·2–22·5)
Macaca mulatta	26	25·8 (22·6–31)
Macaca sinica	2	26·5 (26·2–26·8)
Macaca fuscata	2	27·6 (26·6–28·7)
Cercocebus torquatus	8	25·5 (24–27)
Cercopithecus aethiops	2	26·8 (26–27·7)
Presbytis priamus	2	29·6 (29·2–30·1)
Papio spp.	14	29·7 (25–34)
Cebus frontales	2	31·7 (31–32·5)
Ateles geoffroyi	2	31·3 (30·5–32·2)
Saimiri sciurea	1	30·9
Hapale jacchus	2	34·3 (33·8–34·9)
Tarsius spectrum	2	38·1 (37–39·3)
Perodicticus potto	1	28·3
Daubentonia madagascariensis	2	42·5 (42·3–42·7)
Lorisidae spp.	2	26·2 (26–26·4)
Lemur fulvus nigrifrons	2	34·5 (34·4–34·5)

Daubentonia and gorilla, which alone shows that it furnishes no criterion of demarcation between the lower and the higher apes. In *Pongo* and *Hylobates* it falls to exceptionally low limits, suggesting that in these either the coracoid is exceptionally short or the clavicle exceptionally long. Schultz (1930) states that in *Pongo* the clavicle is unusually long but his figures show that in *Hylobates* it is rather short relative to the length of the humerus.

Table IV

Species	Claviculo-humeral index (Schultz) in degrees	Coraco-clavicular index in degrees	Coraco-humeral index in degrees
Homo	47·7	31·5	15·0
Gorilla	37·1	39·0	14·5
Pan	42·7	34·4	14·7
Pongo	48·1	21·9	10·5
Hylobates	39·0	21·5	8·4
Macaca	39·2	26·6	10·4
Cebus	36·5	31·7	11·6
Ateles	29·1	31·3	7·1
Lemur	43·1	34·5	14·9

In Table IV are set out the values obtained for the claviculo-humeral index, taken from Schultz (1930), the coraco-clavicular index and the coraco-humeral index. From these it appears that in general the coracoid has a more constant relationship to the clavicle than to the humerus. This would be in accord with

the supposition that changes in the mode of progression, and especially the adoption of a brachiating habit, modified the humerus to a greater degree than they did the clavicle, and hardly modified the coracoid at all. But this can only be taken as a very broad and general hypothesis, for many complicating factors are involved in such changes.

A few other points worth noting arose in the course of the work. In man the anterior end of the coracoid is usually rounded, but in many apes, notably the gorilla, it is sharply bevelled, thus making the medial border much longer than the lateral. In some specimens of gorilla also, the under-surface of the acromion is hollowed out into an even concavity which terminates medialwards at a rather sharp point. This is probably due to the upper end of the humerus bearing against the acromion as the animal leans on its knuckles.

SUMMARY

Examination of the coracoid process in man and many other species of Primates reveals that the human coracoid process differs from that of all other Primates examined in some features, notably in the largeness of the upper coracoid angle. Examination of the scapula in a representative series of mammals indicates that the most permanent and invariable part of the bone is the base of the spinous process with the glenoid cavity at its outer extremity.

The coracoid appears to have a more constant relationship to the clavicle than to the humerus. This lends support to the suggestion that changes in modes of progression modify the humerus to a greater extent than the clavicle and hardly modify the coracoid at all.

The bones which we examined are in the National Museum, Dublin; Trinity College, Dublin; Johns Hopkins University, Baltimore, Md.; and McGill University, Montreal. We are very grateful to the authorities at the first three institutions for permission to examine this material.

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THE DEVELOPMENT OF THE ATRIO-VENTRICULAR VALVES IN MAN

By P. N. B. ODGERS

From the Department of Human Anatomy, University of Oxford

The fact that in every text-book of embryology the details of the formation of the atrio-ventricular valves are scanty and that no particular account of their development has appeared since 1876 when Bernays, who used chiefly calves' hearts for his material, published his classical paper, suggested a re-investigation of this matter.

For this purpose I have had at my disposal human embryos of 7·1, 11·2, 11·4, 12·5, 14·5, 15·5, 17, 17·5, 23, 28·5, 46 and 61 mm. crown-rump length. In addition to these the hearts from foetuses of 85, 105, 130 and 210 mm., from two full-time stillborn infants and from a baby of 11 months were specially sectioned for this purpose.

In the detailed description which follows it will perhaps make for clarity to explain that in the tricuspid valve the right cusp in the embryo becomes eventually the inferior or posterior cusp, while the septal cusp becomes the medial one: at the mitral orifice the right cusp of the embryonic heart corresponds to the anterior or aortic cusp, the left one to the posterior cusp of the fully developed valve. Further, it is sometimes convenient to speak of lateral and central cusps. In the former group are included the right cusp of the tricuspid and the left cusp of the mitral valve, while the septal cusp of the tricuspid and the right cusp of the mitral constitute the central cusps.

7·1 mm. EMBRYO

While the superior (dorsal) and inferior (ventral) auriculo-ventricular cushions (hereafter abbreviated to A.-V. cushions) are well marked here, there is no evidence yet of any right or left lateral cushions. There is, however, marked trabeculation of the ventricular wall.

11·2 mm. EMBRYO

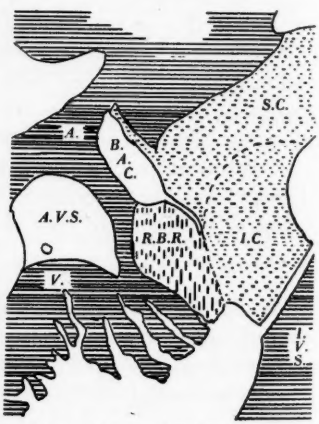
When the right bulbar ridge (or proximal bulbar swelling) is traced in a caudal direction, its left border is seen to join the right side of the superior A.-V. cushion above the level of the right A.-V. orifice. At the level of that orifice the bulbar ridge swells out slightly to become the right lateral cushion (Pl. I, fig. 1). The left lateral cushion also has made its appearance at this stage. It forms a plump projection beneath the endocardium. The positions of these lateral cushions seem to be slightly different. The dorsal half of the right one, where it is at its broadest, is bounded laterally by auricular muscle which separates it from the epicardium occupying the right A.-V. sulcus, while its

ventral half, thinning out in a tail-like fashion, is bordered laterally by the muscular trabeculae of the ventricle. In contrast to this, in the case of the left one, which is roughly quadrangular in shape, its lateral border is in relation to the ventricular wall only, while its upper border is in contact with auricular muscle. In conformity with this the left A.-V. sulcus, which is shallower and much less extensive in all its diameters than the right, is just above the level of the cushion. Both the lateral cushions are already connected with the lateral ventricular walls by muscular trabeculae, the chordae musculares. While the right cusp of the tricuspid valve and the left cusp of the mitral are thus clearly to be distinguished at this stage, there is no evidence of an anterior cusp for the tricuspid valve. The right and left superior and inferior tubercles of the fused A.-V. cushions project prominently on the medial margins of the A.-V. orifices, the sulcus between the tubercles on the left side giving this border a definitely bifid appearance, but there is no other indication of the formation of the central cusps.

11·4 MM. EMBRYO

This specimen is, in my opinion, older than its C.R. length would indicate. The latter was computed by counting the sections and there is no record of its original length.

The left border of the right bulbar ridge (*R.B.R.*) is here seen to have grown across the upper part of the right A.-V. orifice to form the bulbo-auricular channel (*B.A.C.*) and thus to have become contiguous with the right side of the fused A.-V. cushions, first with the superior and then more caudally with the inferior cushion (*I.C.*) (Text-fig. 1). At the same time the ventral border of the bulbar ridge projects above the remainder of the original right A.-V. opening as the anlage of the anterior cusp of the tricuspid valve in which it forms all the cushion tissue. This latter is perforce continuous caudally with the right lateral cushion, both cushions being derivatives of the right bulbar ridge. The right and left lateral cushions still maintain their rather different situations which were noted above in the 11·2 mm. embryo. The right cusp of the mitral valve can now be recognized as a well-marked structure. It is obviously formed by the left extremities of the superior and inferior moieties of the fused A.-V. cushions which project as superior and inferior tubercles.



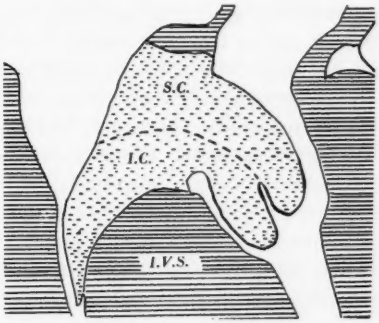
Text-fig. 1. Outline drawing of a section through the heart in an 11·4 mm. embryo (×72). *S.C.* and *I.C.* fused superior (dorsal) and inferior (ventral) A.-V. cushions; *R.B.R.* right bulbar ridge; *B.A.C.* bulbo-auricular channel; *A.* auricular muscle; *V.* ventricular muscle; *A.V.S.* auriculo-ventricular sulcus; *I.V.S.* interventricular septum.

Each of the latter is connected with the lateral wall of the left ventricle by quite a stout band of muscle. At first sight it seems a little difficult to imagine exactly how this occurs, as obviously the trabeculae cannot cross the A.-V. orifice to make contact with the cushion tissue. Each band, however, derived from trabeculae of the ventricular wall, reaches the cushion above and below the left A.-V. orifice and spreads over it, thus forming the anterior and posterior papillary muscles with their chordae musculares. There is no evidence yet of a septal cusp for the tricuspid valve.

12·5, 14·5 AND 15·5 MM. EMBRYOS

These three specimens may be grouped together. In all of them there is little change in the condition of the lateral cusps.

All three, however, show a curiously bilobed appearance in the right cusp of the mitral valve (Text-fig. 2). At 12·5 mm., as in the earlier 11·4 mm. embryo, this is marked only by a notch, but in the later specimens it is much more accentuated. This bilobulation corresponds to the superior and inferior tubercles of the fused cushions. The first indication of the septal cusp of the tricuspid valve occurs in my 14·5 mm. embryo (Text-fig. 2) as a projection from the ventral extremity of the right margin of the inferior A.-V. cushion. This is much more pronounced in the later 15·5 mm. specimen.



Text-fig. 2. Outline drawing of a section through the heart in a 14·5 mm. embryo (×54). *S.C.* and *I.C.* fused superior and inferior A.-V. cushions; *I.V.S.* interventricular septum.

17 AND 17·5 MM. EMBRYOS

The tricuspid valve. In the anterior cusp the base of the flap, which is still for the most part composed of cushion tissue, is being invaded by muscle from the ventricular wall. Strands of muscle from both the anterior and the right ventricular wall appear to meet in a **V**, which separates the cushion tissue from the epicardium in the A.-V. sulcus. In the right cusp (Pl. I, fig. 2) the right lateral cushion, which is much less plump than in the earlier specimens, is in contact with auricular muscle for the greater part of its extent. This separates it from the epicardium of the A.-V. sulcus completely, although at the junction of auricular and ventricular muscle the connexion between the two latter becomes tenuous in some sections. Only the ventral extremity of the cushion borders the ventricular muscle which is forming the valve flap. This muscular flap is much better formed than in the earlier embryos. In

contrast to its appearance in the 11·2 mm. specimen the A.-V. sulcus has now become definitely angulated on the right side. Whether this is caused by an increasing growth of the ventricular wall dorsalwards or no, it must indicate an invagination of the sulcus here. It may be noted that the auricular and ventricular muscle meet each other just dorsal to the attachment of the valve cusp which thus contains no auricular muscle. The differentiation of the septal cusp proceeds slowly. In these embryos there is, however, just ventral to the right ventral border of the A.-V. cushion, commencing trabeculation of the muscular interventricular septum, and some muscle fibres are growing upwards on either side of the projecting spur of cushion tissue. The papillary muscles of this valve can now be distinguished in great part. The anterior one springs from the right wall of the ventricle and is joined at its lower border to the septal wall by a stout moderator band. The anterior papillary muscle is connected with both the anterior and lateral cusps. The posterior papillary muscle comes from the septal wall and joins by its chordae musculares the lateral cusp. The septal papillary muscle is continuous with the posterior one and is very small. It is represented merely by the trabeculation of the septal wall noted above, just ventral to the A.-V. cushion.

The mitral valve. While in the greater part of its extent the right cusp is formed solely by the fused A.-V. cushions, at its cranial and caudal borders it is much more muscular. This is seen best near its caudal end where the chordae musculares of the posterior papillary muscle are continuous on the ventral border of the cushion tissue with the left side of the muscular interventricular septum. There is now no bilobulation of this cusp as was noted in the earlier specimens. The left lateral cushion is rather smaller and somewhat more oval in shape than it was in the earlier embryos. Its relations to the auricular muscle and to that of the ventricular wall show little change. In contrast, however, to the right side, there is here no angulation of the A.-V. sulcus (Pl. I, fig. 3).

23 MM. EMBRYO

(Pl. I, fig. 4)

In this specimen the cushion tissue in the valves with the exception of the left lateral one appears to be at its maximum. It is more marked than in the previous embryo or in any of the later ones. The central cusps (*M.C.*) straddling the dorsal border of the muscular interventricular septum are strikingly prominent. The lateral ones again may be contrasted. On the right side, as in the two previous embryos, the angulation of the A.-V. sulcus is marked and, while the contracted state of the ventricle makes it difficult to define precisely the base of the cusp, I think the auricular muscle joins that of the ventricle just ventral to the line of its attachment. No such angulation is noticed on the left side. In both lateral cusps the cushion tissue seems to have become more ventral. In the case of the right cushion (*R.C.*), its lower two-thirds are in contact with ventricular muscle which is invading its lateral border, while

in the left lateral cusp (*L.C.*), which is almost completely muscular now, the much smaller cushion mass is bordered entirely by it. In both cusps the cushion tissue thus enters much more into the composition of the actual valve flap than it has done previously. Further, it may be noted, that the dorsal portion of the left lateral cushion has altered its character: it has become more cellular and stains more intensely than the rest of it.

28·5 MM. EMBRYO

In contrast to the last specimen the heart of this embryo shows a marked diminution of cushion tissue as a distinct constituent of the A.-V. valves and its replacement by muscle. Further, the cushion tissue itself, as was first noted in the left lateral cusp of the previous embryo, is more cellular and in the case of the two lateral cusps appears as elongated thickenings of the endocardium.

The tricuspid valve. The anterior cusp is almost completely muscularized by muscle which has grown into it from the ventricular wall. There is only a little cushion tissue still remaining on its left margin. The right cusp (Pl. I, fig. 5) is composed for the most part of ventricular muscle with the much attenuated cushion bordering it medially throughout its extent. This cushion tissue, in its lower four-fifths, is in contact with ventricular muscle while in its upper fifth it meets the muscle of the auricle. The junction of the latter with the ventricular muscle is seen to be just ventral to the attachments of the valve cusp. This is much more obvious now than in the previous 23 mm. specimen and is due apparently to an increasing invagination of the right A.-V. sulcus, which was first noted at the 17·5 mm. stage. The base of this cusp at this stage thus consists from within outwards of cushion tissue, auricular muscle, epicardium and ventricular muscle. The septal cusp is better differentiated. There is a small mass of cushion tissue dorsally and laterally but the flap is essentially muscular, muscle strands from the right side of the muscular interventricular septum meeting those which have grown upwards from the chordae musculares.

The mitral valve. The right cusp still contains a good deal of cushion tissue, but it is being muscularized on its ventral border by the muscular chordae which appear to split and to grow along both its ventricular and auricular aspects, while muscle fibres from the left border of the muscular interventricular septum are investing the ventricular surface of the cusp in its dorsal portion. In the left cusp very little cushion tissue remains. It is composed almost entirely of muscle derived from the lateral wall of the ventricle which is continuous with that of the chordae musculares. Neither auricular muscle nor epicardium from the left A.-V. sulcus enter into its composition, for there is still no invagination of the sulcus here as is seen on the right side.

Comparing the different valve cusps at this stage, while there is still much cushion tissue to be seen in the right cusp of the mitral valve and in the septal

cusp of the tricuspid, there is little in the right cusp of the tricuspid, less in the left cusp of the mitral, and the anterior cusp of the tricuspid valve is almost completely muscularized.

46 AND 61 MM. EMBRYOS
(Pl. II, figs. 6, 7)

In both of these all the valve cusps are increasingly muscular. The remains of the cushion tissue have become more difficult to define accurately and appear as elongated strands of cells, which are often broken up into two parts by portions of the cusp entirely muscular, but which are always lining the auricular surface of the flaps immediately beneath the endocardium. As has been noted in the 28·5 mm. embryo, however, this attenuated cushion tissue reaches almost to the free border of the cusps in the 46 mm. specimen, but in the 61 mm. stage it is much less extensive. The angulation of the A.-V. sulcus seen on the right side in the 28·5 mm. embryo can still be found in the 46 mm. one. Here the vertical limb of the sulcus appears as a narrow line separating the lateral border of the most ventral part of the auricular muscle from that of the ventricle (Pl. II, fig. 6), but it differs from the earlier specimen in that it is less extensive and does not enter into the base of the cusp, from which it is always excluded by the junction of auricular with ventricular muscle. As a corollary to this, auricular muscle now takes no part in the composition of the cusp.

At 61 mm. (Pl. II, fig. 7) even the remains of this angulation has gone and the sulcus appears to be straightened out. With this, whether it be *propter hoc* or not, the auricular canal can now be said to have disappeared. In neither of these embryos does the muscle of the auricle extend on to any of the other valve cusps.

85, 105 AND 130 MM. FOETUSES

All the hearts so far examined came from embryos already serially sectioned and stained with haemotoxylin and eosin. In the case of the foetuses now to be considered, the hearts were removed and appropriately sectioned, the sections being stained either by van Gieson or by Masson's trichrome method, which latter was recommended to me by Dr H. M. Carleton. Owing to the plane of the sections the anterior cusp of the tricuspid valve was not examined in these three specimens. All three may be considered together, and the valve cusps in all of them show two remarkable changes when compared with those of the earlier embryos. In the first place, instead of being almost entirely muscular they have become almost completely collagenous, while, secondly, in the case of the lateral cusps this collagenous plate is directly continuous with the epicardium filling the A.-V. sulcus. Actually the collagenous change is most marked in the 85 mm. foetus (Pl. II, fig. 8) where this alteration in character is almost complete in all the cusps except the left lateral one. With

it of course the chordae musculares of the earlier hearts have now become chordae tendineae. What may be cushion remains appear in the more ventral half of the cusps as looser, more cellular, tissue beneath the endocardium. In some of the cusps in the 105 and 130 mm. foetuses there are still some muscle fibres on their auricular aspect: this muscle, as must be concluded from the earlier specimens, is ventricular in origin. There are, however, in both my 85 and 105 mm. foetuses instances of auricular muscle straying on to the valve flap in both cusps of the mitral valve in the former and in both the lateral cusps in the latter specimen. There are sections through the right cusp of the tricuspid valve in the 105 mm. foetus which show auricular muscle extending some distance on to the flap and being continuous with the degenerating ventricular muscle on this aspect of it: in other sections this continuity is interrupted opposite the A.-V. sulcus by collagenous fibres derived from the latter. Sections through the left cusp of the mitral valve in the same heart show quite unequivocally auricular muscle lining this surface of the flap in its upper two-fifths. In all these three hearts, in the case of the lateral cusps, the continuity of the collagenous fibres of the cusp with those of the epicardium in the A.-V. sulcus separates the muscle of the auricle from that of the ventricle. This is in direct contrast to what was constantly found in this situation in all the previous embryos.

210 MM. FOETUS

The valve cusps of this heart are all entirely collagenous, and I do not see any evidence of the remains of cushion tissue. There is a little muscle continuous with that of the ventricular wall to be seen on the ventricular aspect of the upper sixth of the anterior cusp of the tricuspid valve, while in the more central sections of the right cusp auricular muscle strays on to the cusp just beyond its attached border (Pl. II, fig. 9). Nothing is seen of this in the more rostral and in the more caudal sections. In all the other cusps of either the tricuspid or the mitral valves auricular muscle is entirely absent. A comparison of the left lateral cusp of this heart with the same cusp at the 130 mm. stage provides a remarkable contrast.

TWO FULL-TIME STILLBORN INFANTS

There is very little difference to record in the appearance of the valve cusps in these hearts in comparison with my 210 mm. foetus. The annulus fibrosus is, however, thicker and better defined. The right cusp of the tricuspid valve in either heart shows some small broken-up bundles of auricular muscle just beyond its attached border but these are always very trifling (Pl. II, fig. 10), while similarly on the ventricular aspect of its base a very small amount of ventricular muscle can be distinguished. On the other hand, none of the other cusps of any valve in these hearts shows any evidence of auricular muscle in its composition. One must mention in this connexion the right or anterior cusp of the mitral valve. Some of my sections of one of these hearts

show a picture similar to that illustrated in more than one text-book from adult hearts where the muscle of the left auricle is said to be clothing the base of this cusp. I am sure this is not the case in my specimen. There is nothing to indicate accurately the line of attachment of the anterior cusp here, which appears to be continuous with the wall of the aortic vestibule right up to the semilunar valves, except that opposite the base of the left (posterior) mitral cusp the wall of the vestibule tapers to the much thinner valve cusp. If the two mitral cusps, however, are compared (and this is possible here as both cusps appear in the same section), the auricular muscle is found not to extend so far anteriorly as it does posteriorly, i.e. the left A.-V. sulcus is on a line caudal to the limit of the auricular muscle on the posterior aspect of the aortic vestibule. Therefore, to include auricular muscle in the anterior cusp one must make the line of the latter's attachment considerably rostral to that of the posterior cusp and this, so far as I know, has never been suggested.

DISCUSSION

From the above detailed description one can distinguish three definite periods in the formation of these valves. I (from 11·2 to 23 mm.). Here the valve cusps have two distinct components, the cushion tissue and the muscular trabeculae of the ventricular wall, of which the former is the more prominent. II (from 28·5 to 61 mm.). In these hearts the cusps are apparently essentially muscular with only the remains of the cushion tissue on their auricular aspect. III (from 85 mm. to full term). In this period the muscle in the cusps is replaced entirely by collagenous tissue. These different stages are shown in Pls. I and II in the case of the right lateral cusp.

I. From 11·2 to 23 mm. While the dorsal and ventral A.-V. cushions are well-marked structures in my 5 mm. specimen, the lateral cushions do not appear in my series until the 11·2 mm. stage (Pl. I, fig. 1). Waterston (1918) found both right and left lateral cushions in his 8 mm. embryo, while Mall (1912) described the presence of the left lateral one at 9 mm., "but both are not well pronounced until the embryo is 11 mm. long". Such a diagram as Walmsley (1929) draws of a transverse section through the atrial canal, which shows it surrounded completely by a continuous ring of cushion tissue with the A.-V. cushions unfused, is clearly misleading. The right bulbar ridge (Frazer, 1931) or proximal bulbar swelling (Tandler, 1913) is certainly continuous in my 11·2 mm. embryo, as Waterston noted, with the right lateral cushion. Whether, as he interpreted it, the latter should be regarded as entirely formed by a downward prolongation of this ridge or whether there is an independent subendocardial proliferation with which the ridge gains continuity, as Mall suggested, is a difficult question to decide. Although in my 11·2 mm. specimen the greatest transverse diameter of the cushion slightly exceeds that of the ridge above it, I am inclined to agree with Waterston and to regard it as formed wholly from the bulbar ridge. Certainly, too, the right bulbar ridge, as is

seen in my series from the 11·4 to the 17·5 mm. stage, forms the cushion tissue of the anterior cusp of the tricuspid valve. As Frazer (1916–17) first showed clearly, although both Mall and Waterston suggested it, the right bulbar ridge grows across the tricuspid orifice (Text-fig. 1) and joins by its left border the fused A.-V. cushions, while its free margin bulges ventrally and overhangs the caudal remaining portion of the tricuspid opening (of which the rostral part is forming the bulbo-auricular channel) to persist as the cushion basis of the anterior cusp of this valve. The right bulbar ridge is thus seen to be responsible for the formation of both the anterior and the right, or posterior, cusps of this valve and their common origin explains the continuity between them which is seen in the adult heart. In the stages I am now considering, this continuity is such that one can only distinguish between the two cusps in serial sections by the patency or otherwise of the A.-V. canal. Further, the primitive fusion of the bulbar ridge with the A.-V. cushions and subsequently with the proliferation from the latter, which fills the caudal ventral portion of the interventricular foramen (Odgers, 1937–8), accounts for the attachment of the anterior cusp medially in the adult heart to the membranous part of the septum where it meets and becomes continuous with the anterior border of the septal cusp.

The valve cusps appear in the following sequence: While both the lateral cusps are beginning to be well formed at the 11·2 mm. stage, the right, or anterior, cusp of the mitral valve is rather later in its appearance: it is a well-marked structure in my 11·4 mm. embryo. The anterior cusp of the tricuspid can just be recognized in the same embryo, but is not well formed until the 14·5 mm. stage is reached. The last one to appear is the septal cusp of the tricuspid: this is first indicated clearly in the 15·5 mm. specimen in my series. While both superior (dorsal) and inferior (ventral) A.-V. cushions enter into the composition of the right mitral cusp as is indicated by its bilobulation noted in the 12·5, 14·5 and 15·5 mm. embryos (Text-fig. 2), the cushion tissue of the septal cusp of the tricuspid valve is formed only from the inferior one. Granted that its accurate definition is always difficult, it is probably correct to say that the right cusp of the mitral valve has the largest amount of cushion tissue in its composition and the left one the least of all the valve cusps.

I should again insist on the position and relations of the lateral cushions in this stage of their development. They are subendocardial and are entirely separated on their lateral side from the epicardium filling the A.-V. sulcus by auricular muscle joining that of the ventricle, while their ventral halves are in contact with the muscular trabeculae of the ventricular wall.

II. 28·5, 46, and 61 mm. embryos (Pl. I, fig. 5; Pl. II, figs. 6, 7). In the three embryos included in this group one finds the muscular element in the valve cusps becoming more and more predominant. The muscle gradually invades and replaces the cushion tissue. This invasion appears to proceed at the base of the cusp from ventricular muscle derived in the case of the lateral cusps from the lateral wall and in that of the central ones from the muscular

interventricular septum. (In this connexion it is interesting to note that the A.-V. cushions themselves are largely invaded by this same muscle in these later embryos and it is possible, although I have not been able to confirm this with my present material, that they go through a muscular stage like the valves and like them later become collagenous. At all events this proliferation would account for the presence of muscle fibres in the pars membranacea septi in the adult heart (Walmsley).) At the same time, on the ventral borders of the central cusps the chordae musculares grow up on either side of the cushion tissue: this is perhaps seen better in the case of the right cusp of the mitral valve. The remains of the cushion tissue appear to migrate ventralwards. This migration is difficult to explain. It is clearly seen to occur if the microphotographs of the right lateral cusp of the 17·5 and the 46 mm. embryos are compared (Pl. I, fig. 2 and Pl. II, fig. 6). In the former the upper end of the cushion is in contact with auricular muscle, while in the latter it is all ventral to this, and instead of being confined to the base its remains extend almost to the free border of the cusp. I think this migration must be due to the active proliferation of the cushion tissue, and this would account for its more cellular appearance.

Is there, at this stage, an increasing invagination at the A.-V. sulcus? Waterston found in the hearts of his 28·6 and 30 mm. embryos that the right lateral cusp showed an increasing infolding at the A.-V. sulcus so that at this stage the cusp consists, near its base, of a core of fibrous tissue continuous with the epicardium of the sulcus with auricular muscle on its medial and with ventricular muscle on its lateral surface. Tandler, whose description is not easy to follow, would seem to give a like account. I should first perhaps define as precisely as possible what I take to be the upper limit of the cusp. This is not always easy, as it varies in different sections with the presence or absence in any particular section of the most medial row of chordae musculares. I take, however, for this purpose a line at right angles to the long axis of the cusp at the most ventral point of its basal attachment to the ventricular wall. This corresponds in most cases to that given by Gross & Kugel (1931), although their method of determining it was a different one. If with this definition in mind the microphotograph from my 17·5 mm. embryo (Pl. I, fig. 2) is compared with that from my 28·5 mm. one (Pl. I, fig. 5), there does seem to be a commencing invagination, marked by its angulation, at the right A.-V. sulcus in the former embryo, which has so far increased in the later specimen that the junction of auricular and ventricular muscle is seen to be ventral to the line of the attachment of the cusp. The result of this is that epicardium does invade the uppermost quarter of the right lateral cusp, and this portion of it is thus precisely like Waterston's description. There is nothing like this in the case of the corresponding left lateral cusp, of the composition of which Waterston makes no mention at this stage. In my embryos this consists solely of ventricular muscle with a small amount of cushion tissue on its medial aspect in its upper portion. Here one may note that the left A.-V.

sulcus is much less deep than the right one throughout my series. From my 11·2 mm. embryo up to my 61 mm. one, the proportion is roughly 63 : 100 as average figures. The microphotograph of the 23 mm. embryo shows this contrast (Pl. I, fig. 4) which must be correlated with the thicker wall of the right ventricle. In the later embryos of this same stage, 46 and 61 mm., the anatomy of the right lateral cusp is again different. Here the angulation of the A.-V. sulcus gradually disappears and the auricular muscle stops abruptly well above the level of the cusp attachment so that its junction with the ventricular muscle prevents any epicardium from entering the base of the valve, which consists essentially of ventricular muscle with some remains of cushion tissue on its medial aspect (Pl. II, figs. 6, 7). The condition seen in my 28·5 mm. embryo must be a transitory phase. That it is not exceptional is proved by Waterston finding it the same in his 28·6 and 30 mm. specimens. It is, too, this temporary condition of the right lateral cusp which must account for such a description of the development of these valves as Keith (1933) gives. "The auricular canal with an attenuated envelopment derived from the ventricle thus comes to hang within the ventricular chambers and forms the lateral cusps of the tricuspid and mitral valves. The morphological demarcation between auricle and ventricle lies at the free edge of the A.-V. valves." Even for this stage such an account would seem to be a great exaggeration, and, while it may be substantially true for the pig's heart at 10 mm., it finds no confirmation, in my opinion, in either the earlier or the later stages of the development of these valves in the heart of man.

III. Foetuses of 85, 105 and 210 mm., and at full time. As has been seen above, two changes occur simultaneously at this stage. The muscular flap becomes increasingly collagenous. As Benninghoff (1930) remarks, it is uncertain if one should speak of the degeneration and replacement of the muscle or of its transformation into fibrous tissue. Probably the former would be more in accord with accepted views. With this change the collagenous cusp in the case of the lateral ones becomes continuous with the epicardium filling the A.-V. sulcus. This confirms Bernays' (1876) original statement: "Die Vereinigungsstelle zwischen Klappen und Pericardialfortsatz wird also sehr spät gebildet, sie entspricht dem 'annulus fibrocartilagineus' der Autoren." The effect of this is that, while up to this time, i.e. up to about 4 months, auricular muscle has met that of the ventricle, this junction is now for the first time interrupted. At 6½ months, which corresponds to my 210 mm. stage, the cusps are all completely collagenous and there is no further change in their essential anatomy.

In view of the differences of opinion about the condition of the adult valves, it is interesting to note here the presence or absence of muscle in the later stages of their development now being considered. There is, in some sections of the lateral cusps at different ages, a small amount of ventricular muscle just arching over their attached borders to reach the uppermost portion of the cusp on its ventricular aspect. This seems to occur quite capriciously. In my

85 mm. foetus it is present in both lateral cusps, in my 210 mm. specimen it is absent in both, but it does occur in the anterior cusp of the tricuspid valve, while at full term it is present in the right lateral cusp but is absent in the left one. With regard to the presence of auricular muscle in the cusps, again in this series of foetal hearts its appearance is quite irregular. I have found it just overlapping the base of the cusp, according to the definition of the latter I have enunciated already, in the following instances: at 85 mm. both the mitral cusps show that auricular muscle just overlaps their attached borders in certain sections; in my 105 mm. specimen auricular muscle occurs in both lateral cusps and is particularly well marked in the left one; in the heart of the 210 mm. embryo some sections of the right lateral cusp show its presence, while at full term in many sections of the same cusp it is still seen but is less extensive (Pl. II, figs. 9, 10). Lastly, in the heart of an 11 months old baby it is found in some sections of the left lateral cusp. In no other valve cusp in this series is there any evidence of auricular muscle taking any part in its constitution, so that it is found only in some sections of seven out of a possible thirty-two cusps. I agree with Bernays that it is not found much before the fifth month, but I have found no evidence that it spreads more and more on to the surface of the valves in the later months of foetal life as he insisted. He correlated the presence of auricular muscle in the cusps with their increasing growth. The valves grow, he wrote, from the periphery and draw with them in their growth auricular muscle and the endocardium covering it; the extension of this muscular layer must go hand in hand with the growth of the cusp. The question of an increasing infolding at the A.-V. sulcus in these later foetal stages is a difficult one to answer, as the sulcus naturally deepens with the increasing size of the heart. Certainly in the case of the right lateral cusp in my 210 mm. specimen there is not only an increase in the depth of the sulcus but it is angulated, and this is more marked again at full term. I believe that this alteration could be simply explained as being wholly due to the growth of the ventricular wall in both a dorsal and a lateral direction. Further, I do not see that the presence of auricular muscle need be now compulsorily associated with increasing infolding or cusp growth (in contrast to the much earlier 28·5 mm. stage) as it is now divorced from that of the ventricle by the continuity of the collagenous matrix of the cusp with the A.-V. sulcus. This view is confirmed if the corresponding left lateral cusp is considered. Here there is no change either in the depth or in the direction of the A.-V. sulcus like that seen on the right side until full term is reached, and yet at 105 mm. there is a remarkable extension of auricular muscle on to it. I have already referred fully to the exceptional condition found in the right lateral cusp in my 28·5 mm. embryo. Waterston did not examine any later stage than that seen at 30 mm., so that it is hardly correct for him to say in his final discussion of this question that "the structure of the infolded cusp is a centrally wedge-shaped mass of connective tissue derived from the external surface of the A.-V. junction with a layer of muscle tissue continuous with the atrial muscle coat on the atrial

surface and of ventricular muscle substance on the ventricular aspect". Benninghoff described the composition of a lateral cusp in a 12·4 cm. embryo as consisting of auricular muscle, epicardium from the primitive coronary sulcus and the cortical and trabecular muscle of the ventricle. The illustration of a reconstruction of this cusp, which he gives and which is presumably the left lateral one, differs very much from any of my sections, while his definition of the cusp includes the medial two-thirds of the A.-V. sulcus. (Probably 12·4 cm. should read 12·4 mm., but even so I cannot agree with his description.) From this he concludes, as Tandler (1913) does, that "die Entwicklung der Atrioventrikularklappen lässt es verständlich erscheinen dass ausser den Sehnenfäden auch die Klappenmembran zu beiden Seiten Reste von Herzmuskulatur nahe der Klappenwurzel enthalten Kann".

My own observations would not support this view. The appearance of auricular muscle in the valve cusps in the series that I have examined is so infrequent and so irregular that I cannot correlate it with any increasing infolding at the A.-V. sulcus. Nor would this method account for its occurrence in the central cusps, where no such invagination can take place. I would suggest rather that its presence in the adult heart valves should be looked upon as an irregular proliferation which is as capricious as it is in the foetal heart and for which there is no constant embryological foundation.

The fate of the endocardial cushions

In the earlier stages (Pl. I, figs. 1–3) only the more ventral portion of the cushions can be said to be actually forming part of the cusps. Later, as is seen in my 28·5, 46 and 61 mm. embryos (Pl. I, fig. 5; Pl. II, figs. 6, 7), the remains of the cushion tissue appear to have proliferated as elongated strands of cells beneath the endocardium which extend almost to the free border of the cusps in the case of the first two, but are much less well marked in the third member of this group. At 85 mm. (Pl. II, fig. 8) and at 130 mm. the greater part of the cusps are collagenous, but there is still in their ventral halves a looser, more cellular tissue beneath the endocardium. I think one should regard these areas as cushion remains. Bernays suggested that the nodules of Albini represent their last survival, but in my 210 mm. specimen the whole cusp appears to be homogeneously collagenous. There does seem thus to be evidence that some remains of the endocardial cushions do survive their invasion by muscle and do not disappear until the whole valve cusp is entirely collagenous.

CONCLUSIONS

1. The development of the atrio-ventricular valve cusps falls into three stages: (*a*) From 11·2 to 23 mm. they are composed of endocardial cushion tissue and muscle derived from the ventricular trabeculae. The right bulbar ridge provides the basis of cushion tissue for the anterior and right cusps of the tricuspid valve. (*b*) From 28·5 to 61 mm. they are becoming increasingly

muscular, while the remains of cushion tissue can be still distinguished on their auricular surfaces. (*c*) From 85 to 210 mm. they gradually become entirely collagenous and there is no further change in their composition.

2. Except for the temporary appearance presented by the right cusp of the tricuspid valve near its base at 28·5 mm., in no stage in this series are the cusps regularly composed of auricular and ventricular muscle separated by a wedge of connective tissue. The occasional presence of auricular muscle seems to be entirely capricious.

3. There is therefore no constant embryological foundation for the occurrence of auricular muscle in the valves of the adult heart.

I have to thank Prof. E. Fawcett and Prof. D. M. Blair for the loan of embryos from their collections which amplified our own series here, and Mr W. Chesterman of this department for the preparation of the microphotographs.

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EXPLANATION OF PLATES I AND II

Plate I

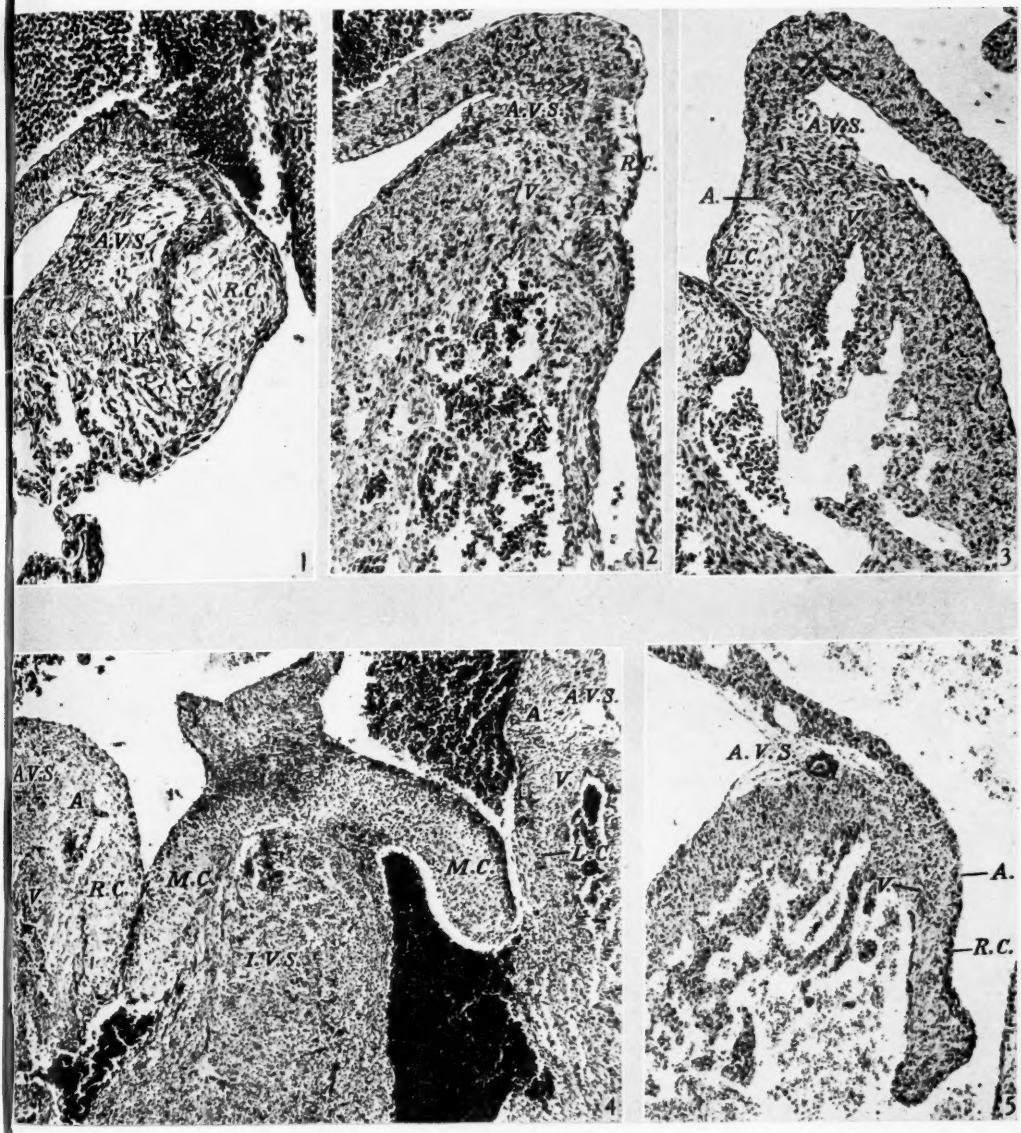
Fig. 1. A section through the right A.-V. orifice in an 11·2 mm. embryo (× 109). It shows the right lateral cushion, *R.C.*, separated from the A.-V. sulcus, *A.V.S.*, by auricular muscle, *A.*, joining that of the ventricle, *V.*

Fig. 2. A section of the right lateral cusp in a 17·5 mm. embryo (× 109) to show the angulation of the right A.-V. sulcus, *A.V.S.*, which is still separated from the right lateral cushion, *R.C.*, by the junction of auricular, *A.*, with ventricular muscle, *V.*

Fig. 3. A section through the left lateral cusp in the same embryo as fig. 2 (× 109) to show in contrast the absence of any angulation of the sulcus, *A.V.S.*, while the left lateral cushion, *L.C.*, maintains its original shape and relations to *A.*, auricular muscle and to *V*,, ventricular muscle.

Fig. 4. A section through the heart in a 23 mm. embryo (× 60). It shows the cushions of the central cusps, *M.C.*, capping the muscular interventricular septum, *I.V.S.*, and those of the lateral cusps, *R.C.* and *L.C.* Note the angulation of the right A.-V. sulcus, *A.V.S. A.* auricular, *V.* ventricular muscle.

Fig. 5. A section through the right lateral cusp in a 28·5 mm. embryo (× 60) to show the effect of the invagination which has now occurred at the right A.-V. sulcus, *A.V.S.* Auricular muscle, *A.*, joins that of the ventricle, *V.*, ventral to the line of attachment of the cusp, so that its base is now composed of auricular muscle, *A.*, and ventricular muscle, *V.*, with a core of connective tissue continuous with that of the sulcus. The cushion tissue, *R.C.*, extends almost to the tip of the cusp.



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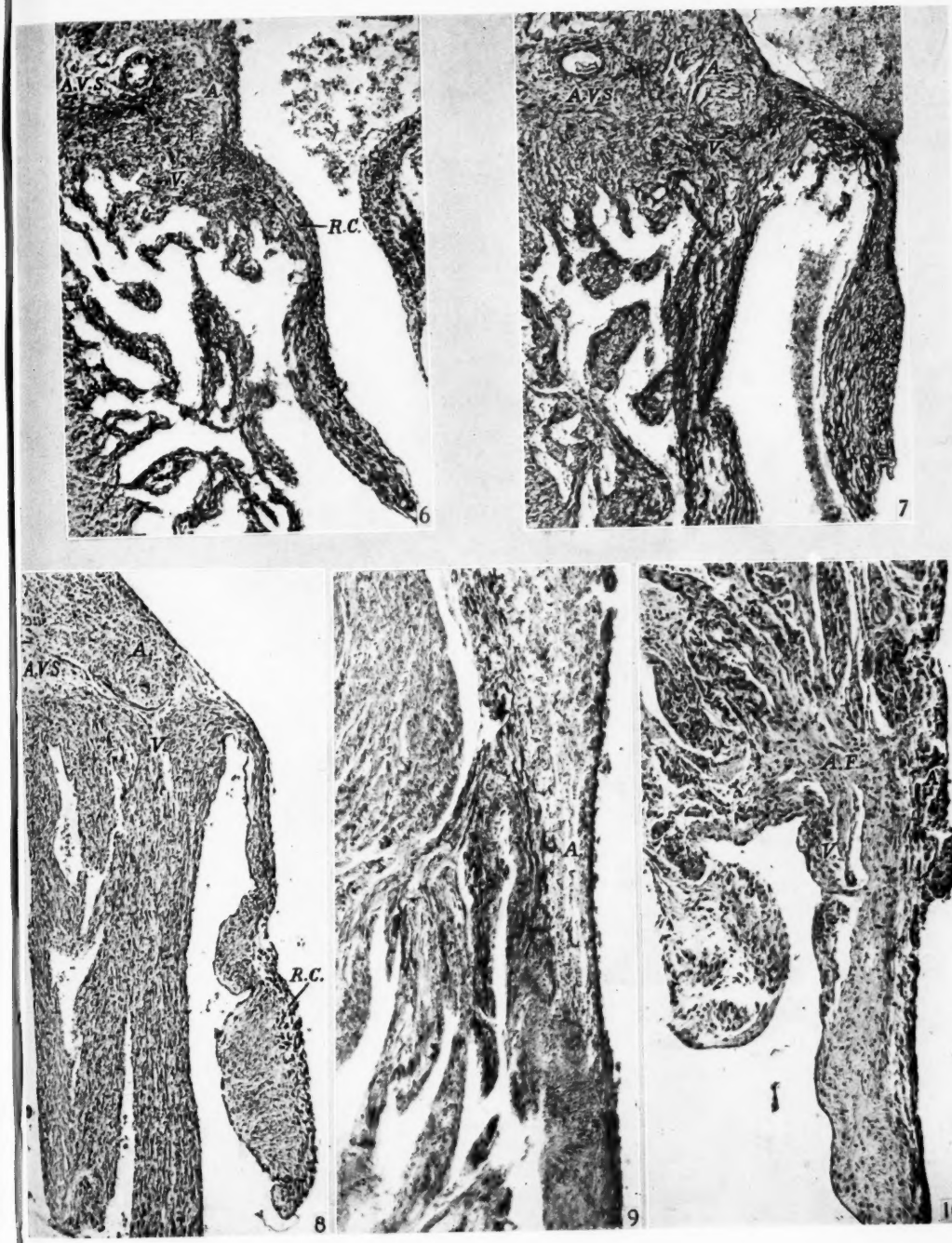

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PLATE II

Fig. 6. A section through the right lateral cusp in a 46 mm. embryo (× 60). Here the cusp consists entirely of ventricular muscle, *V.*, with the remains of the right cushion, *R.C.* The junction of auricular muscle, *A.*, with that of the ventricle, *V.*, is now dorsal to the line of attachment of the cusp and the angulation of the sulcus, *A.V.S.*, is much less marked.

Fig. 7. A section through the right lateral cusp in a 61 mm. embryo (× 60). The angulation of the sulcus, *A.V.S.*, has now disappeared and, as before, auricular muscle, *A.*, joins that of the ventricle, *V.*, dorsal to the base of the cusp.

Fig. 8. A section through the right lateral cusp in an 85 mm. foetus (× 60). The cusp is now collagenous and is continuous with the A.-V. sulcus, *A.V.S.*, this continuity separating auricular muscle, *A.*, from that of the ventricle, *V.* Ventricular muscle just comes over on to the base of the cusp. *R.C.* marks the apparent remains of cushion tissue.

Fig. 9. A section through the right lateral cusp in a 210 mm. foetus (× 60) to show that auricular muscle, *A.*, does extend on to the base of the cusp in some sections at this stage, the rest of which is entirely collagenous.

Fig. 10. A section through the right lateral cusp of a full time foetus (× 60). Note the well-marked annulus fibrosus *A.F.*, and that both ventricular muscle, *V.*, and auricular muscle, *A.*, stray on to the base of the cusp to a very small extent.

THE CONDITION OF THE PERITONEAL VAGINAL PROCESSES AT BIRTH

By G. A. G. MITCHELL

Department of Anatomy, Aberdeen University

It is commonly stated that the peritoneal vaginal processes do not close until after birth, but in the course of an investigation into the spread of intraperitoneal effusions, in which still-born infants were used for experimental purposes, it was found that this belief is not always correct. The experiments necessitated the injection of a fine barium emulsion into the peritoneal cavity through artificial visceral perforations, and the mode of spread was studied by X-rays. During the greater part of the experiment the pressure was kept low, but at the end high pressures were employed to test the patency or otherwise of the vaginal processes. After each experiment the infants were dissected in order to check the radiographic findings, and the ossific centres in the femora, tibiae, and tarsal bones were examined.

The condition of the processes was investigated in forty cases, twenty-six male and fourteen female. Nineteen of the male and ten of the female infants were mature, as judged by the presence of centres of ossification in the lower ends of the femora and in the cuboids. The others, seven male and four female, were all near maturity, as only larger infants were selected for experimental purposes. Four infants (one male and three female) which showed centres of ossification in the upper ends of the tibiae are not included in the series, even though this is not definite evidence of post-maturity. In all twenty-six male cases the testes were either present in the scrotum or could be pressed into the scrotum without difficulty. None of the specimens showed ectopia testis.

In the following table the term "completely patent" means that the process showed no evidence of constriction at any point (Fig. 1); "completely closed" means that the process was completely separated from the general peritoneal cavity by obliteration of the process near the deep inguinal ring (Fig. 2); and by "partial obliteration" is meant a distinct narrowing in the same region (Fig. 3). It is well established that narrowing of the process usually begins above and extends downward toward the testis, and in this series there was only one specimen in which closure occurred between the funicular and testicular parts before the process as a whole became separated from the general peritoneal cavity.

The table on page 3 shows that in three male and four female cases the processes were completely closed on both sides *by the time of birth*, that in one male and three female cases the process was completely closed on

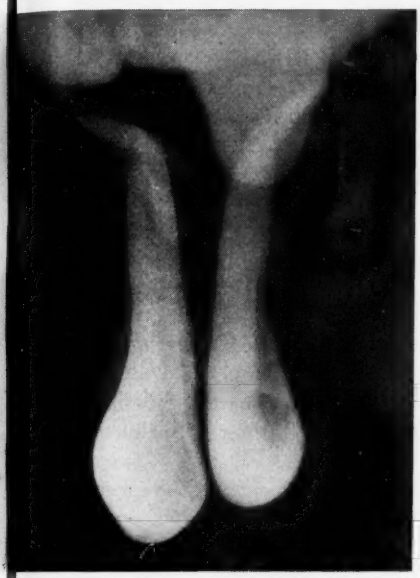

Fig. 1.

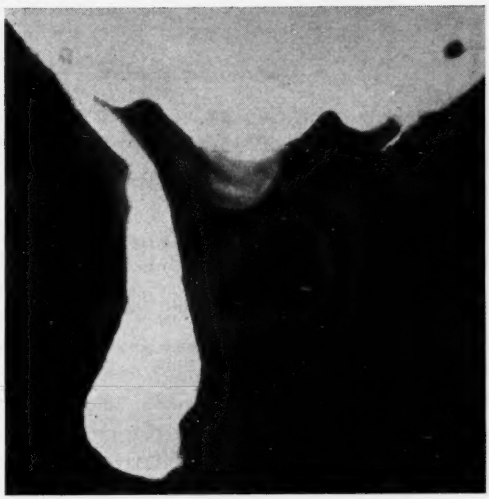

Fig. 3.

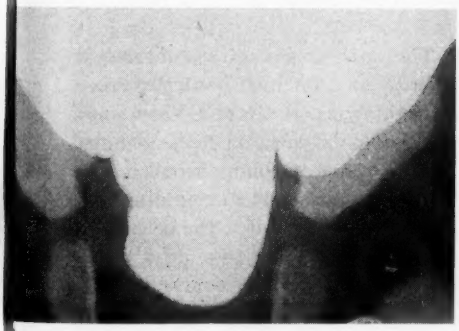

Fig. 2.

Fig. 4.

Fig. 1. Complete bilatera patency of the peritoneal vaginal processes.
Fig. 2. Complete bilateral closure of the peritoneal vaginal processes.
Fig. 3. A specimen showing complete patency of the right and partial obliteration of the left vaginal processes.
Fig. 4. The arrow indicates the small femora peritonea diverticulum mentioned in the text.

one side and partly obliterated on the other, and that in one male case it was completely closed on one side and completely patent on the other.

Condition of peritoneal vaginal processes	Mature		Immature	
	M.	F.	M.	F.
Completely patent on both sides	7	2	3	0
Completely patent on one side and partly obliterated on opposite side	2*	0	2*	0
Completely patent on one side and completely closed on opposite side	1*	0	0	0
Funicular part of process closed below but open above	1	0	0	0
Partly obliterated on both sides	4	3	2	2
Partly obliterated on one side and completely closed on opposite side	1	2	0	1
Completely closed on both sides	3	3	0	1

* In all these cases the completely patent processes were on the right side.

Many authorities might be quoted in support of the belief that the processes do not become separated from the general peritoneal cavity until after birth, but only a few representative opinions need be given.

Keibel & Mall (1912): "After the completion of the descensus the saccus vaginalis remains connected with the abdominal cavity by a narrow canal. A short time after birth this becomes at first solid and is then resorbed. The saccus vaginalis, now completely cut off from the abdominal cavity, has become the tunica vaginalis propria testis."

Arey (1934): "Within a few weeks to a few months after birth the narrow canal which connects the processus vaginalis with the abdominal cavity becomes solid and its epithelium is resorbed. The vaginal sac, now isolated, represents the tunica vaginalis of the testis."

Keith (1933): "*Processus Vaginalis*—The processus vaginalis becomes occluded by adhesion or zygosis at two points soon after birth, but in a considerable proportion of individuals the process of closure is delayed. The upper point of occlusion takes place at the internal abdominal ring; the lower a short distance above the testicle. The part of the processus vaginalis between the points of occlusion is known as the *funicular process*; the part surrounding the testicle becomes the tunica vaginalis. In quite 30 % of children the occlusion takes place at the internal abdominal ring some considerable time after birth or it fails altogether. Occlusion may fail at the upper point, at the lower point, or at both. Or it may close at both points, but the funicular process, instead of disappearing, may remain open and form a cyst."

Frazer's (1937) account alone appears to indicate the possibility of closure by the time of birth. He states that the changes in the vaginal process are as follows: "(1) About the period of birth the vaginal process usually becomes constricted and closed superiorly at the deep inguinal ring, and then the process is an elongated tube, closed at each end, its lumen being now shut off from the cavity of the peritoneum. (2) A few days after birth the process

usually becomes constricted and closed a little above the testis. (3) The funicular portion of the process usually becomes impervious and converted into a fibrous thread, which as a rule disappears in the course of the first month after birth. (4) The testicular portion of the process persists as a shut serous sac, called the tunica vaginalis."

One specimen showed an interesting state of affairs on the right side (Fig. 4). Two small pouches appeared, and in order to determine their relationship to the vessels the vascular system was injected, but unfortunately all the vessels showed up well except those of the right lower limb. It was found on dissection, however, that the inner pouch projected for a short distance into the femoral canal, while the outer pouch represented the upper end of a partly obliterated processus vaginalis. Small centres of ossification were found in the lower ends of both femora which felt distinctly gritty on section, though they were apparently too small to cast a detectable X-ray shadow. The infant must therefore have been about full-time, and the presence of this femoral peritoneal diverticulum was interesting, as it lends support to the view that there is a congenital basis even for some femoral herniae.

SUMMARY

1. A table is supplied showing the condition of the peritoneal vaginal processes at birth in forty infants.

2. It is pointed out that in certain cases the process is obliterated on one or both sides in the region of the deep inguinal ring *before birth.*

3. A specimen is described in which a small peritoneal femoral diverticulum was found.

I am deeply indebted to Prof. A. Low for having allowed me, over a period of years, to use the X-ray equipment in the Anatomy Department, Aberdeen University, and for giving me the benefit of his valued criticism and advice. The expenses of the investigation have been defrayed with the aid of grants from the Medical Research Council.

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ANATOMICAL NOTE

NOTE ON A CASE OF PATENT DUCTUS ARTERIOSUS AND PATENT FORAMEN OVALE IN A MATURE SHEEP

By F. J. R. BEATTIE
Department of Physiology, Chelsea Polytechnical College, London

AND

W. R. M. MORTON
Department of Anatomy, Cambridge

A NUMBER of cases of non-closure of the ductus arteriosus in various mammals have been reported (Giepel, Schmutzer, Hare and Orr, Segall and Mirsky), but little is known about the histology of the non-closed ductus in mature animals. Recent work on the normal physiological closure of the ductus arteriosus in sheep by Barclay, Barcroft *et al.* (1938) indicates that it is a rapid phenomenon occurring almost immediately after delivery. Boyd (1937) has shown that there is a marked histological difference between the structure of the ductus arteriosus and the adjacent parts of the pulmonary artery and aorta. This difference, which consists of a marked predominance of smooth muscle fibres in the ductus arteriosus as compared with the relatively non-muscular condition of the other two vessels, suggests that the structure of the former is the anatomical basis of its sudden constriction at birth, i.e. that its apparently physiological closure, as contrasted with anatomical obliteration, is due to contraction of this musculature. It would be very interesting, therefore, to know if any difference in the structure of the wall of the ductus arteriosus, e.g. paucity of muscle, is present in those cases where there is non-closure in the adult.

The following note is a record of the histological findings in such a case, with some observations on these findings. The material consisted of the heart and vessels of a mature sheep, which was obtained by one of us (F. J. R. B.) for routine class work, so that over 24 hr. elapsed before the vessels were fixed in formalin. There was no history of the condition of the sheep during life. The heart shows a patent foramen ovale, guarded by a very well developed sleeve-like valve which projects into the left auricle for a distance of 2 cm. There is a well-marked patent ductus arteriosus, 0·9 cm. in length, running from the pulmonary artery (from a point just proximal to its bifurcation) to the aorta. The ductus arteriosus continues distally for a further 0·5 cm., incorporated in the aortic wall, before opening into the aortic lumen. The relevant vessels were sectioned and stained for muscle, elastic and collagen fibres. The material did not take the differential stains well, owing to the post-mortem changes which had occurred before fixation.

The aorta shows relatively few bundles of smooth muscle fibres as compared with the number seen in a normal sheep aorta. The muscle fibres are scattered throughout the thickness of the wall instead of being arranged in well-marked bundles occupying the outer half or two-thirds of the wall. The elastic tissue and collagen seems to be present in about equal amounts, and they constitute the main bulk of the wall. The pulmonary trunk shows slightly more muscle tissue than the aorta, but it is not conspicuous. There is no sign of bundle formation as in the normal pulmonary trunk, which shows considerably more muscle bundles than the normal aorta. Elastic tissue is present, but not so much as in the aorta. Collagen fibres make up the bulk of the wall. The ductus arteriosus shows a very well-marked internal elastic lamina, immediately outside which were about 50–60 layers of muscle, collagen and

elastic tissue, arranged concentrically. There appears to be more muscle tissue in the outer layers than in the inner layers, and a greater proportion of muscle in the ductus than in the aorta. Surrounding the outside of the ductus is a very thick layer of elastic fibres.

The probable course of the blood was as follows: Some of the blood from the right atrium passed into the right ventricle and thence to the pulmonary artery as in the normal heart, but some must have passed through the foramen ovale into the left atrium. It is inconceivable that blood passed from the left atrium into the right atrium as the valve guarding the foramen ovale was so complete. Mixed blood therefore entered the left ventricle and aorta. Judging by the large size of the pulmonary trunk prior to its junction with the ductus arteriosus, and its subsequent reduction in size (we cannot use the post-mortem direction of the ductus arteriosus as a guide to its direction during life (Barclay *et al.*)), it seems reasonable to assume that blood passed from the pulmonary artery to the aorta. This is contrary to what might be expected from a consideration of the normal pressure differences between the two vessels, and also from the assumption that the venous stream has already been divided into two. Considering the structure of the ductus in this case, there is no persistence of the condition present in the ductus of a newly born lamb, but rather there is the appearance of a structure designed to withstand high pressures. Whether this is due to the altered pressure changes in the vessels owing to the cardiac abnormality, or to the absence of the normal amount of smooth muscle at birth (leading to non-closure of the ductus and hence back pressure on the right side of the heart, with consequent failure of closure of the foramen ovale), it is not possible to say.

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Anatomical Society of Great Britain and Ireland

PROCEEDINGS

February, May 1939

RECORDED BY

A. J. E. CAVE

PROCEEDINGS. ANATOMICAL SOCIETY

FEBRUARY 1939

An Ordinary Meeting of the Society was held in the Department of Anatomy, University of Leeds at 10 a.m. on Saturday, 18 February 1939, with Professor A. B. APPLETON, Vice-President, in the Chair.

Professor F. WOOD JONES read a paper on "The anterior superior alveolar nerve and vessels", which will be submitted for publication.

Professor A. DURWARD read a paper on "The origin of the corpus striatum", which will be submitted for publication.

Dr UNA FIELDING regarded the conclusions put forward as simplifying current conceptions of old and new cortical fields and as clarifying a rather confused issue in neurology. Professor APPLETON discussed the origin of the conception of cortico-striate continuity and Professor BLAIR enquired concerning the developmental origin of the claustrum. Replying, Professor DURWARD remarked that by some authorities the histological appearances in the adult brains of certain forms had been unwarrantably interpreted as evidence of an embryological continuity of cortical and striate fields; embryological material, when examined, failed to demonstrate any such continuity: the claustrum was generally regarded as being formed by secondary cortical delamination.

Messrs D. STEWART and W. LEWINSKY read a paper on "The innervation of the gum of *Talpa europea*", which will be submitted for publication.

Replying to Dr WEDDELL, the authors stated that they had not observed a genuine neural network within the mole's tactile discs, although such an arrangement was known to be present in other species, and that they did not deny the possibility of the association of two distinct types of nerve ending with a single nerve fibre.

Messrs F. WOOD JONES and V. F. LAMBERT read a paper on "The occurrence of the lemurine form of ectotympanic in a primitive marsupial", which will be submitted for publication.

Professor APPLETON enquired as to the possible physiological effects of the intrabullar disposition of the ectotympanic upon the tympanic membrane. Dr GREEN detailed the condition of parts in the bulla of *Phascolomys* and discussed the corresponding structural arrangements in *Centetes*, wherein the basisphenoid also participates in the formation of the auditory bulla. Dr CAVE regarded the lemurine encapsulation of the ectotympanic as a primitive, non-primate character of distinct taxonomic importance. Professor WOOD JONES recounted the disposition of the ectotympanic element in the skulls of various mammalian groups, with special reference to the components of the auditory bulla: no information was as yet available concerning any specialized functional activity on the part of the ear drum in forms manifesting the intrabullar type of ectotympanic.

Dr R. W. HAINES read a paper on "The evolution of secondary centres in epiphyses", which will be submitted for publication.

Professor BRASH welcomed the recognition of growth cartilage and articular cartilage as distinct epiphysial components, a conception which accorded well with observed phenomena in the growth of individual bones. He asked whether cell column formation had been noted on both sides of the growth cartilage of long bones,

and was answered in the negative. Mr RICHARDSON asked whether the quantity and quality of the material examined was sufficient to exclude those specific or dietary variations known to affect calcification. Dr MACCONAILL enquired whether any particular attention had been devoted to the blood supply of secondary centres. In reply, Dr HAINES detailed the sources and the condition of preservation of the material used, from which the variations mentioned could be eliminated: in view of the many excellent investigations of previous workers he had not dealt particularly with details of epiphysial vascularity.

Dr W. E. ADAMS read a paper on "Observations on the avian heart".

Examination of the heart of *Apteryx australis* (the New Zealand kiwi) has confirmed the fact that it is typically avian in structure, as affirmed by Lankester and Beddard; and, further, that the specialized connexions between the chambers of the heart follow very closely the plan established for other birds by Ohmori and Davies. The principal atrio-ventricular connection, and the subsidiary systems, in so far as they were isolated, correspond essentially to those in other birds. In the kiwi, however, the sino-atrial region presents certain differences, dependent probably upon the more primitive nature of the sinus venosus, which in this species is very simple in form and presents a distinctive musculature.

Professor DAVIES discussed this communication with particular reference to the avain sinu-atrial node, the musculature of sinus and of atrium, and other structural particulars. The present findings in the kiwi heart confirmed previous observations made in other avian species and formed a valuable addition to the anatomy of the conducting system of the heart. The growth of knowledge of this system was surprisingly recent. Less than thirty years ago the occurrence of any specialized conducting tissue in the bird's heart had been denied by high authority: in the interim the anatomy of the system had been worked out in various forms. One distinct type of cardiac conducting system obtained in cold-blooded, and another in warm-blooded, forms.

Dr W. F. HARPER read a paper on "The histology of new blood vessel formation in heart valves", which will be submitted for publication.

Dr G. A. G. MITCHELL read a paper on "The condition of the peritoneal vaginal processes at birth", which will be submitted for publication.

Professor APPLETON suggested a future investigation of the correlation of time closure of the vaginal processes with foetal maturity as assessed by the degree of skeletal development. Replying to Professor DAVIES the author said that the testes were scrotal, or almost so, in all cases, that no instance of ectopia testis occurred in the series, and that in one case only had closure occurred between the funicular and testicular parts of the process before the latter became separated from the general peritoneal cavity. Professor BEATTIE and Dr MACCONAILL also discussed this communication.

Dr D. STEWART read a paper on "The effect of the complete removal of a muscle on the gait of the rabbit", which will be submitted for publication.

Professor APPLETON discussed the effects of muscle division upon the stretch reflex: Dr GREEN enquired whether differences of gait had been noticed following section of red and of white muscles respectively; Professor BLAIR asked whether the removal of the fascia of the muscles concerned affected gait in any way. Dr STEWART replied that the rabbit's soleus was a typically red muscle; no distinction had been observed between division of white and division of red muscles: the muscles selected were all fusiform and experimentally the fascial element could be ignored.

Messrs P. BACSICH and G. WYBURN read a paper on "Observations on the oestrous cycle of the guinea-pig", which will be submitted for publication.

Dr BACSICH stressed the need for accurate determination of the time limits of the cycle and of their variations: at first such variations in length of cycle had been regarded with suspicion, but had later proved to be quite natural. There was no question of any vitamin deficiency in the diet of the animals used.

Dr R. J. O'CONNOR read a paper on "The effects of obstructing the growth of the pronephric duct in *Amblystoma tigrinum*", which will be submitted for publication.

In reply to Dr GRAY the author stated that, in the few species it was possible to consider, there was, in development, some suggestion of a correspondence between the degree of dependence of the optic lens on the optic cup and the degree of dependence of the mesonephros on the presence of the pronephric duct.

Mr R. B. ZACHARY (introduced by Professor DURWARD) read a paper on "Nathaniel Highmore".

Nathaniel Highmore was one of the later figures to come into prominence during the revival of learning in England. His studies at Christ's College, Oxford, proceeded under difficulties, particularly in the sphere of anatomy, and mainly because of the scarcity of anatomical material. He was greatly influenced by Harvey, whose conception of the circulation of the blood he vigorously championed in his *Corporis Humani Disquisitio Anatomica* (1651). In this work occurs the classical reference to the maxillary sinus to which, later, his name became eponymously attached.

Professor BEATTIE referred to Highmore's reputation as a lithotomist and to his portrait in the Royal College of Surgeons. Dr CAVE mentioned the unusual size of the antrum described by Highmore: this cavity is well figured by Leonardo da Vinci and was familiar to Galen himself. Professor BRASH also commented favourably upon this paper.

DEMONSTRATIONS

At this Meeting of the Society the following Demonstrations were given:

(1) A. DURWARD and W. E. ADAMS: A live specimen of *Sphenodon punctatum*.

(2) A. DURWARD and W. E. ADAMS: The brain of *Sphenodon punctatum*.

(3) J. WHILLIS: A case of ovarian pregnancy.

(4) W. F. HARPER: Specimens illustrating new vascular formation in cardiac valves.

(5) W. F. HARPER: Blood supply of the ureter in the cat and the full term human foetus.

(6) G. A. G. MITCHELL: Dissections of autonomic nerves.

(7) T. W. GRIFFITH (introduced by A. DURWARD): Specimen of divided left atrium.

(8) R. OFFER (introduced by A. DURWARD): Early anatomical and medical books in the Leeds University Library.

MAY 1939

An Ordinary Meeting of the Society was held in the Institute of Anatomy and Embryology, University College, London, at 3 p.m. on Friday, 5 May 1939, with the President (Professor ALEXANDER LOW) in the Chair.

Before opening the scientific business of the Meeting, the President announced the presentation of a silver coffee service to Professor E. Barclay Smith, as some slight recognition of his long and valued services in the interests of the Society. He asked Sir Arthur Keith to make the formal presentation.

Sir Arthur Keith: I gladly respond to our President's request that I should express on your behalf, the high esteem in which we hold Professor Barclay Smith, the affection with which we regard him, and the debt which this Society owes him. Our Society, as you know, was brought into existence in 1887, its foundation owing much to its first Secretary, the intrepid and courageous young surgeon of St Bartholomew's Hospital, C. B. Lockwood. But if it was Lockwood who founded the Society, it was the man we honour to-day who established it and brought it to its present sound and flourishing state.

Professor Barclay Smith and I are now contemporary senior members, for we joined in 1893 when the Society was already six years old. Parsons, Wilson, Withers and Fawcett can claim to be senior to us. Only one of the original members remains with us, Professor R. W. Reid of Aberdeen, the doyen of British anatomists. None of us senior members can claim to have worked so persistently and so unostentatiously for the welfare of the Society as the man to whom we do honour to-day.

When in the later years of the Great War he came from Cambridge to take service in the University of London, he threw himself whole-heartedly into the affairs of our Society and for nineteen years was its pilot as well as its Secretary-Treasurer. He established himself as the friend not only of the Society but also of its every member. He brought in the team spirit; his tact and rectitude did the rest. We regret that it is a continuation of his illness that has kept our friend from being with us to-day. But we have a welcome representative here in the person of his distinguished daughter, Mary Barclay Smith. We therefore beg her to take home with her a report of our warm feelings towards her father, our earnest hopes of his return to health, and this more tangible token of our regard—which I have now the pleasure, in your name, of entrusting to her. We thank her for her presence with us to-day.

Miss Mary Barclay Smith: Mr President, ladies and gentlemen, I know you share with me my deep regret that my father cannot be with you in person to receive this kind gift and to hear the gracious and moving words of its presentation. I can assure you, however, that now, as indeed at all Meetings of the Anatomical Society, his thoughts are with you. I know too that all his work for the Society was to him a source of the greatest pleasure and interest, and that no words of mine can express his gratitude, not only for this gift which he will treasure always, but also for the great loyalty and affection shown him by all members of the Society during his years of office. I am charged to convey to you his hopes for the future of the Society. May it proceed from strength to strength in the spirit of loyal co-operation that has distinguished it in the past, that your contributions to human knowledge may be of ever increasing value. Mr President, members of the Society, on behalf of my father, I thank you.

Mr J. A. HARPMAN read a paper on behalf of himself and the late Professor H. H. WOOLLARD on "The connexions of the inferior colliculus and of the dorsal nucleus of the lateral lemniscus" which will be submitted for publication in the *Journal of Anatomy*.

Professor LE GROS CLARK, discussing this paper, asked whether experimental

injury to spino-tectal fibres could be excluded and whether the role of the inferior colliculus as a relay station precluded its operation as a reflex centre. Mr HARPMAN replied that no fibres could be traced from the inferior colliculus into the tecto-spinal tract and that no question of their injury arose.

Dr G. WEDDELL read a paper on behalf of himself and the late Professor H. H. WOOLLARD on "The anatomy of pain", which will be submitted for publication in the *Journal*.

Dr POSENER discussed the communication from the pharmacological and clinical aspect. Professor LE GROS CLARK was informed that no evidence had been observed of anatomical continuity between the terminals of adjacent but distinct nerve fibres.

Messrs J. A. HARPMAN, D. G. LAMBLEY and G. WEDDELL read a paper on "The innervation of the intrinsic musculature of the tongue", which will be submitted for publication in the *Journal*.

Professor BOYD discussed this paper and suggested an extension of the investigation to include such animals as possess a persistent Froriep's ganglion, which is absent in the rabbit and rat.

Drs G. WEDDELL and K. POSENER read a paper on "Studies on the motor nerve-supply of foetal muscle" which will be submitted for publication in the *Journal*.

Dr G. R. DE BEER read a paper on "The origin of visceral arch cartilages from the neural crest". Numerous experiments performed during the last few years, involving extirpation and transplantation of neural crest in Amphibia, can only be interpreted on the view that the visceral arch cartilages (except the second basibranchial) and the trabeculae are derived from the cells of the neural crest. That this is the case in normal development has also been claimed by some workers, but without supplying convincing evidence in the form of figures. A matter of such wide general importance appeared worthy of definite confirmation, which is now provided by means of a detailed study of the histogenesis of axial and visceral cartilage in the axolotl. The pigment and the yolk contained in ectodermal and mesodermal derivatives respectively, has been traced into the actual cartilage of the visceral arches (except the second basibranchial) and trabeculae on the one hand and of the axial cartilage on the other.

Professor J. P. HILL remarked upon the importance of this, the first direct ontogenetic evidence of the derivation of the arch cartilages from neural crest cells. From his own earlier work on the development and fate of the neural crest in the marsupial embryo he had come to regard so-called head mesoderm as really mesectoderm, deriving from the paramedullary proliferation. Whether the whole of this paramedullary proliferation became mesectoderm remained uncertain: Sir ARTHUR KEITH welcomed the paper as likely to provide a satisfactory basis for the explanation of such puzzling malformations as agnathia, cyclops, defective Meckelian cartilage development, and the like.

Messrs K. J. FRANKLIN, A. E. BARCLAY, D. H. BARRON and Sir JOSEPH BARCROFT exhibited a cinematograph film of "Movements of the valve of the foramen ovale" in the heart of the newly born sheep.

Dr J. H. GRAY read a paper on "Experiments on the growth and regenerative capacity of lymphatic trunks" which will be submitted for publication in the *Journal*.

Dr WHILLIS commented on the influence of scar tissue upon the development of sprouts from the divided ends of lymphatic trunks: he suggested that the evidence submitted did not exclude the influence of lymph-flow upon the sprouting of new lymphatic vessels. Dr GRAY, in reply, remarked that whilst sprouting occurs from

both ends of a divided lymph trunk the newly formed lymph-vessels always dispose themselves parallel to the scar: the influence of lymph-flow upon lymphatic regeneration was admittedly not settled by the present experiments, but it should be remarked that, whilst both ends of a severed lymphatic trunk manifest sprouting, only one of the ends can be directly influenced by the lymph current.

Dr R. S. Hawes (introduced by G. R. De Beer) read a paper on "The structure of the eye in *Proteus anguineus* Laur." The eye of *Proteus anguineus* lies beneath the skin and is invisible, in the adult, from the surface. It is surrounded by a sclera, in which no cornea is differentiated, and choroid. The pigmented epithelium lacks "pseudopodial" processes. The usual layers of the retina can be counted. The optic nerve is a stump unconnected with the brain. The space which would be occupied by a lens and vitreous humour in a normal Urodelan eye is, in the adult *Proteus*, filled by a cellular mass proliferated from the ciliary zone. The so-called integumentary cornea of Schlampp does not exist. So far, only two extrinsic eye muscles have been seen in sections of adult heads; previous workers have asserted that all six or none at all are present.

Replying to Mr Harpman the author stated that, correlated with its degenerative optic apparatus, *Proteus* exhibits a much reduced mesencephalon, probably associated with the complete absence of all tracts concerned with visual impressions.

Messrs W. E. Le Gros Clark and S. Sunderland read a paper on "Structural changes in the isolated visual cortex" which will be published in the *Journal*. Mr Harpman recalled Woollard's finding of the normal histological visual cortical pattern in the congenitally anophthalmic dog and Monakow's similar finding after ocular extirpation. The visual cortical pattern appeared to depend upon the interaction of its immediate afferent and efferent connexions. Professor Le Gros Clark in reply mentioned the curious persistence of the oculomotor nucleus after third nerve section and the inexplicably divergent degree of neural atrophy produced experimentally in the lower visual centres of different animal species.

Dr P. N. B. Odgers read a paper on "The development of the atrio-ventricular valves in man" which will be submitted for publication in the *Journal*.

Dr W. Brandt read a paper on "The nasal cavity in a 124 mm. human foetus" which will be submitted for publication in the *Journal*.

The communication was discussed by Professor Harris, Drs Cave and Haines.

Messrs W. B. Griffiths and E. C. Amoroso read a paper on "The placenta of the Blackbuck (*Antilope cervicapra*), with remarks on some features in the placentation of the cow, sheep and goat" which will be submitted for publication in the *Journal*.

Messrs E. C. Amoroso, W. B. Griffiths and W. J. Hamilton read a paper on the "Tubal journey and rate of cleavage of the goat's ovum" which will be submitted for publication in the *Journal*.

Messrs E. C. Amoroso, W. B. Griffiths and W. J. Hamilton read a paper on "A living tubal ovum of the horse". The recovery of a living tubal ovum of the horse is reported. Its morphological characters are described and the measurements of the intact egg in isotonic salt solution and after fixation are given.

Demonstrations

At this Meeting of the Society the following Demonstrations were given:

(1) C. S. Hallpike and W. E. Peet: A modification of wax-plate reconstruction technique.

(2) G. Weddell, J. A. Harpman and L. Young: Histological preparations of the innervation of (*a*) the tongue, (*b*) the skin.

(3) G. Weddell: Method for stimulating and recording action potentials from nerves in the rabbit's ear.

(4) J. H. Gray: Techniques for investigation of lymphatic vessels.

(5) R. J. O'Connor: Some points in the technique of experimental embryology.

(6) W. Brandt: Model of nasal cavity in 124 mm. human foetus.

(7) F. Goldby: Degeneration in cortico-spinal and other tracts in *Echidna aculeata*.

(8) P. Ford (introduced by G. R. De Beer): Modification of existing technique for fixation of vital dye in serially sectioned material and a method for counter-staining the sections.

(9) K. C. Richardson: The cytology of experimental diabetes in dogs with reference to the islets of Langerhans.

(10) K. C. Richardson: The Crossman-Mallory triple stain.

REVIEWS

A Dictionary of Scientific Terms. By I. F. Henderson and W. D. Henderson. 3rd edition, revised by J. H. Kenneth. London: Oliver and Boyd. 1939. Price 16*s*.

The third edition of this dictionary is very welcome. It is revised by J. H. Kenneth who was actually responsible for beginning the work which appeared first in 1920. Ill-health, however, prevented him from continuing it at that time. The dictionary is clear, concise and accurate. It contains definitions of over ten thousand terms, and the pronunciation and derivation of each term are also given. It should be emphasized that the work is confined to the biological sciences, and for this reason the title of the dictionary might perhaps be more explicit. With modern developments in scientific research, involving the breaking down of barriers which have previously separated, rather artificially, one department of biological science from another, this dictionary will become more and more an essential part of the equipment of the biological worker.

The Morphology of the Brachial Plexus. By Wilfred Harris, M.D. Oxford University Press; London: Humphrey Milford. 1939. Price 25*s*.

This book is mainly a record of the dissections of the brachial plexus in vertebrates. The text is accompanied by numerous diagrams showing the composition of the plexus in each type examined. As a work of reference, the book will prove useful to the comparative anatomist. Its value is somewhat limited, however, by the fact that individual variations are hardly taken into account. The zoologist is likely to be irritated by certain taxonomic errors, such as the reference to the "higher mammalian classes", and to the "lowest group" of the Primates as consisting of "Lemurs, Lemuroids, and Marmosets". The bibliography is surprisingly scanty in view of the extensive literature in which references to the brachial plexus are to be found.

The Post-natal Development of the Human Cerebral Cortex. Vol. I. *The Cortex of the Newborn.* By L. Le Roy Conel. Harvard University Press; London: Humphrey Milford. 1939. Price 35*s*.

This volume embodies a descriptive account of the cytoarchitecture of the cerebral cortex of the new-born infant, based on the study of six specimens. It is illustrated by 98 photographic plates, all of which are beautifully reproduced. The main value of the book is that it provides an atlas for reference. It will appear to some readers that it has been published at a time when anatomists are becoming less interested in cytoarchitecture as the morphological expression of cortical organization, for the recent work of Lorente de Nó and others has tended to emphasize the importance of synaptic connexions rather than the laminar arrangement and morphology of cell bodies in the cortex. We would question the value of including the microphotographs of Cox-Golgi preparations, for the camera lucida drawings from these preparations are sufficient to illustrate the cell types. The text is detailed and comprehensive. It is somewhat surprising, however, to read that in the cortex of the new-born infant no Nissl bodies and no neurofibrils are present in any cells.

The author is to be commended for this painstaking study. Subsequent volumes will no doubt provide the opportunity for the general conclusions which follow from an arduous investigation of this kind.

Anatomy of the Human Lymphatic System. By H. ROUVIÈRE, translated by M. J. TOBIAS. (Ann Arbor, Michigan; Edwards Brothers, Inc.) 1938. Pp. viii+318, 129 figs. Price $4.00.

This translation of Prof. Rouvière's well-known *Anatomie des Lymphatiques de l'Homme* provides a welcome addition to our knowledge of this subject. It contains an exhaustive and critical account of the lymphatic nodes and their fields of drainage. It is to be noted that instead of writing of "regional" lymph nodes, the author prefers to describe them as "nodes of the first echelon", because, as he very rightly points out, the term "regional" indicates that the node belongs to one region only, while in fact the nodes of a particular region do not receive all the lymph vessels of a neighbouring organ and may receive also the drainage of several other organs. The chapters on the lymphatics of the head and neck and on those of the thorax seem to us the most outstanding (as might perhaps be expected from the fact that it is on these regions that Rouvière himself has done so much work). Most anatomists will regret that the chapter on their general anatomy is so short. Here such questions as the involution of lymph nodes and the lymphatics of bone, of muscle and of nerves, are touched upon all too briefly; nor is there anywhere any discussion of the comparative merits of the different techniques which have been used for their investigation.

The script and illustrations call for some criticism. In order to keep the price of the work down, photolithographs of perfect typescript replace print. This, with the double-column page, makes the book a tiring one to read in continuity. Further, while the simpler line-drawings are clear and most informative, the more elaborate illustrations are in many cases poorly reproduced and are not very helpful. There is, however, an excellent bibliography of 768 references, and an appendix of variations and historical notes which is curiously called a Glossary.

IN MEMORIAM

L. H. DUDLEY BUXTON, M.A., D.Sc. (Oxon.), F.S.A.
Fellow of Exeter College and Reader in Physical Anthropology in the University of Oxford

The death of Dr Dudley Buxton at the early age of 49 came as a great shock to his wide circle of friends and removed not the least able of the little band of physical anthropologists in this country. Before the War, immediately after taking his degree, he was excavating in Cyprus and in the Sudan, and after it an Albert Kahn Travelling Fellowship enabled him to have a year in the Far East. The results of that year's travel were incorporated in two books, *The Eastern Road* and *Primitive Labour*. For the last nineteen years he had been teaching physical anthropology in Oxford and was appointed the Reader in this subject in 1928. Of his many papers published during this period, perhaps the more notable are "Man's nasal index in relation to certain climatic conditions" with the late Prof. A. Thomson, his account of the Neanderthal Child's skull from Devil's Tower, Gibraltar, with Dr Dorothy Garrod, and "The essential craniological technique" with Dr G. M. Morant.

About nine years ago first the University, then his College and lastly the city of Oxford, discovered his abilities as a man of affairs, and his many duties as a College Bursar and as the Custodian of the University Parks interfered in some measure with his research work. He has, however, read two papers to this Society recently on "Platymeria and platycnemia" and on "The relation of finger-nail growth to nutritional status". Of late years the statistical method of approach to his subject had increasingly attracted him and he leaves a book, very shortly to be published, entitled, *Measurement and Chance in Biology*. From this brief consideration of his published work it will be at once apparent that Buxton, far from being a narrow specialist, was a man of wide interests and of large and varied knowledge, with a ready pen and great powers of precise exposition. An abiding memory of him among his many friends will be his constant and cheerful readiness to help wherever his aid was sought. However occupied he might be with his own work—and his many activities always kept him busy from morning to night—he always found time to listen patiently, his opinion was never given hastily, and it was seldom unimportant.

P. N. B. O.

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